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(71) Applicant (for all designated States except US): **BAYER AKTIENGESSELLSCHAFT [DE/DE]; 51368 Leverkusen (DE).**

(72) Inventor; and

(75) Inventor/Applicant (for US only): **RAMAKRISHNAN, Shyam [IN/US]; Apartment #10, 76 Euston Road, Brighton, MA 02135 (US).**

(74) Common Representative: **BAYER AKTIENGESSELLSCHAFT; 51368 Leverkusen (DE).**

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(54) Title: **REGULATION OF HUMAN GALANIN RECEPTOR-LIKE G PROTEIN COUPLED RECEPTOR**

(57) Abstract: Reagents which regulate human galanin receptor-like GPCR and reagents which bind to human galanin receptor-like gene products can be used to regulate the effect of galanin for therapeutic purposes. Treatment of pathophysiological disorders such as eating disorders, cancer, diabetes, osteoporosis, obesity, pain, depression, ischemia, Alzheimer's disease, sleep disorders, migraine, anxiety, and reproductive disorders can be treated. Processes such as cognition, analgesia, sensory processing (olfactory, visual), processing or visceral information, motor coordination, modulation of dopaminergic activity, and neuroendocrine function can be modulated.

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REGULATION OF HUMAN GALANIN RECEPTOR-LIKE G PROTEIN COUPLED RECEPTOR

5 TECHNICAL FIELD OF THE INVENTION

The invention relates to the area of G-protein coupled receptors. More particularly, it relates to the area of galanin receptor-like G protein coupled receptors and their regulation for therapeutic purposes.

10

BACKGROUND OF THE INVENTION

G-Protein Coupled Receptors

15 Many medically significant biological processes are mediated by signal transduction pathways that involve G-proteins (Lefkowitz, *Nature* 351, 353-354, 1991). The family of G-protein coupled receptors (GPCR) includes receptors for hormones, neurotransmitters, growth factors, and viruses. Specific examples of GPCRs include receptors for such diverse agents as dopamine, calcitonin, adrenergic hormones,
20 endothelin, cAMP, adenosine, acetylcholine, serotonin, histamine, thrombin, kinin, follicle stimulating hormone, opsins, endothelial differentiation gene-1, rhodopsins, odorants, cytomegalovirus, G-proteins themselves, effector proteins such as phospholipase C, adenylyl cyclase, and phosphodiesterase, and actuator proteins such as protein kinase A and protein kinase C.

25

GPCRs possess seven conserved membrane-spanning domains connecting at least eight divergent hydrophilic loops. GPCRs (also known as 7TM receptors) have been characterized as including these seven conserved hydrophobic stretches of about 20 to 30 amino acids, connecting at least eight divergent hydrophilic loops. Most
30 GPCRs have single conserved cysteine residues in each of the first two extracellular loops, which form disulfide bonds that are believed to stabilize functional protein

structure. The seven transmembrane regions are designated as TM1, TM2, TM3, TM4, TM5, TM6, and TM7. TM3 has been implicated in signal transduction.

5 Phosphorylation and lipidation (palmitylation or farnesylation) of cysteine residues can influence signal transduction of some GPCRs. Most GPCRs contain potential phosphorylation sites within the third cytoplasmic loop and/or the carboxy terminus. For several GPCRs, such as the β -adrenergic receptor, phosphorylation by protein kinase A and/or specific receptor kinases mediates receptor desensitization.

10 For some receptors, the ligand binding sites of GPCRs are believed to comprise hydrophilic sockets formed by several GPCR transmembrane domains. The hydrophilic sockets are surrounded by hydrophobic residues of the GPCRs. The hydrophilic side of each GPCR transmembrane helix is postulated to face inward and form a polar ligand binding site. TM3 has been implicated in several GPCRs as
15 having a ligand binding site, such as the TM3 aspartate residue. TM5 serines, a TM6 asparagine, and TM6 or TM7 phenylalanines or tyrosines also are implicated in ligand binding.

GPCRs are coupled inside the cell by heterotrimeric G-proteins to various intra-
20 cellular enzymes, ion channels, and transporters (see Johnson *et al.*, *Endoc. Rev.* 10, 317-331, 1989). Different G-protein alpha-subunits preferentially stimulate particular effectors to modulate various biological functions in a cell. Phosphorylation of cytoplasmic residues of GPCRs is an important mechanism for the regulation of some GPCRs. For example, in one form of signal transduction, the
25 effect of hormone binding is the activation of the enzyme, adenylate cyclase, inside the cell. Enzyme activation by hormones is dependent on the presence of the nucleotide GTP. GTP also influences hormone binding. A G-protein connects the hormone receptor to adenylate cyclase. G-protein exchanges GTP for bound GDP when activated by a hormone receptor. The GTP-carrying form then binds to
30 activated adenylate cyclase. Hydrolysis of GTP to GDP, catalyzed by the G-protein

itself, returns the G-protein to its basal, inactive form. Thus, the G-protein serves a dual role, as an intermediate that relays the signal from receptor to effector, and as a clock that controls the duration of the signal.

5 Over the past 15 years, nearly 350 therapeutic agents targeting 7TM receptors have been successfully introduced onto the market. This indicates that these receptors have an established, proven history as therapeutic targets. Clearly, there is a need for identification and characterization of further receptors which can play a role in preventing, ameliorating, or correcting dysfunctions or diseases including, but not
10 limited to, infections such as bacterial, fungal, protozoan, and viral infections, particularly those caused by HIV viruses, pain, cancers, anorexia, bulimia, asthma, Parkinson's diseases, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders,
15 including anxiety, schizophrenia, manic depression, delirium, dementia, several mental retardation, and dyskinesias, such as Huntington's disease and Tourett's syndrome.

Galanin and Galanin Receptors

20 The neuropeptide galanin and its receptors hold great promise as targets for the development of novel therapeutic agents. Galanin is widely distributed throughout the peripheral and central nervous systems and is associated with the regulation of processes such as somatosensory transmission, smooth muscle contractility, hormone
25 release, and feeding (for review, see Bartfai *et al.*, 1993). In the periphery galanin is found in the adrenal medulla, uterus, gastrointestinal tract, dorsal root ganglia (DRG), and sympathetic neurons. Galanin released from sympathetic nerve terminals in the pancreas is a potent regulator of insulin release in several species (Ahren and Linskog, 1992; Boyle *et al.*, 1994), suggesting a potential role for galanin in the
30 etiology or treatment of diabetes. High levels of galanin are observed in human and rat anterior pituitary where galanin mRNA levels are potently upregulated by

estrogen (Vrontakis *et al.*, 1987; Kaplan *et al.*, 1988). The presence of galanin in the hypothalamic-pituitary-adrenal axis coupled with its potent hormonal effects has led to the suggestion that galanin may play an integral role in the hormonal response to stress (Bartfai *et al.*, 1993).

5

Within the CNS galanin-containing cell bodies are found in the hypothalamus, hippocampus, amygdala, basal forebrain, brainstem nuclei, and spinal cord, with highest concentrations of galanin in the hypothalamus and pituitary (Skofitsch and Jacobowitz, 1985; Bennet *et al.*, 1991; Merchenthaler *et al.*, 1993). The distribution of galanin receptors in the CNS generally complements that of galanin peptide, with high levels of galanin binding observed in the hypothalamus, amygdala, hippocampus, brainstem and dorsal spinal cord (Skofitsch *et al.*, 1986; Merchenthaler *et al.*, 1993; Bartfai *et al.*, 1993). Accordingly, agents modulating the activity of galanin receptors would have multiple potential therapeutic applications in the CNS. One of the most important of these is the regulation of food intake. Galanin injected into the paraventricular nucleus (PVN) of the hypothalamus stimulates feeding in satiated rats (Kyrkouli *et al.*, 1990), an effect which is blocked by the peptide galanin antagonist M40 (Crawley *et al.*, 1993). In freely feeding rats, PVN injection of galanin preferentially stimulates fat-preferring feeding (Tempel *et al.*, 1988); importantly, the galanin antagonist M40 administered alone decreases overall fat intake (Leibowitz and Kim, 1992). These data indicate that specific receptors in the hypothalamus mediate the effects of galanin on feeding behavior, and further suggest that agents acting at hypothalamic galanin receptors may be therapeutically useful in the treatment of human eating disorders.

25

Galanin receptors elsewhere in the CNS may also serve as therapeutic targets. In the spinal cord galanin is released from the terminals of sensory neurons as well as spinal interneurons and appears to play a role in the regulation of pain threshold (Wiesenfeld-Hallin *et al.*, 1992). Intrathecal galanin potentiates the anti-nociceptive effects of morphine in rats and produces analgesia when administered alone (Wiesenfeld-Hallin *et al.*, 1993; Post *et al.*, 1988); galanin receptor agonists may

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therefore be useful as analgesic agents in the spinal cord. Galanin may also play a role in the development of Alzheimer's disease. In the hippocampus galanin inhibits both the release (Fisone *et al.*, 1987) and efficacy (Palazzi *et al.*, 1988) of acetylcholine, causing an impairment of cognitive functions (Sundstrom *et al.*, 1988).

5 Autopsy samples from humans afflicted with Alzheimer's disease reveal a galaninergic hyperinnervation of the nucleus basalis (Chan-Palay, 1988), suggesting a role for galanin in the impaired cognition characterizing Alzheimer's disease. Together these data suggest that a galanin antagonist may be effective in ameliorating the symptoms of Alzheimer's disease (see Crawley, 1993). This
10 hypothesis is supported by the report that intraventricular administration of the peptide galanin antagonist M35 improves cognitive performance in rats (Ogren *et al.*, 1992). Human galanin receptors thus provide targets for therapeutic intervention in multiple CNS disorders.

15 High-affinity galanin binding sites have been characterized in brain, spinal cord, pancreatic islets and cell lines, and gastrointestinal smooth muscle in several mammalian species, and all show similar affinity for ¹²⁵I-porcine galanin (about 0.5-1 nM). Nevertheless, recent *in vitro* and *in vivo* pharmacological studies in which fragments and analogues of galanin were used suggest the existence of multiple
20 galanin receptor subtypes. For example, a galanin binding site in guinea pig stomach has been reported that exhibits high affinity for porcine galanin (3-29) (Gu, *et al.* 1995), which is inactive at CNS galanin receptors. The chimeric galanin analogue M15 (galantide) acts as antagonist at CNS galanin receptors (Bartfai *et al.*, 1991) but as a full agonist in gastrointestinal smooth muscle (Gu *et al.*, 1993). Similarly, the
25 galanin-receptor ligand M40 acts as a weak agonist in RINm5F insulinoma cells and a full antagonist in brain (Bartfai *et al.*, 1993a). The pharmacological profile of galanin receptors in RINm5F cells can be further distinguished from those in brain by the differential affinities of [D-Tyr²]- and [D-Phe²]-galanin analogues (Lagny-Pourmir *et al.*, 1989). The chimeric galanin analogue M35 displaces ¹²⁵I- galanin
30 binding to RINm5F membranes in a biphasic manner, suggesting the presence of multiple galanin receptor subtypes, in this cell line (Gregersen *et al.*, 1993).

Multiple galanin receptor subtypes may also co-exist within the CNS. Galanin receptors in the dorsal hippocampus exhibit high affinity for Gal (1-15) but not for Gal (1-29) (Hedlund *et al.*, 1992), suggesting that endogenous proteolytic processing may release bioactive fragments of galanin to act at distinct receptors. The rat pituitary exhibits high-affinity binding for ^{125}I -Bolton and Hunter (N-terminus)-labeled galanin (1-29) but not for ^{125}I -Tyr²⁶-porcine galanin (Wynick *et al.*, 1993), suggesting that the pituitary galanin receptor is a C-terminus-preferring subtype. Spinal cord galanin binding sites, while similar to those in brain, show an affinity for the chimeric peptide antagonist M35 intermediate between the brain and smooth muscle (Bartfai *et al.*, 1991), raising the possibility of further heterogeneity.

A galanin receptor cDNA was recently isolated by expression cloning from a human Bowes melanoma cell line (Habert-Ortoli *et al.*, 1994). The pharmacological profile exhibited by this receptor is similar to that observed in brain and pancreas, and on that basis the receptor has been termed GALR1. The cloned human GALR1 receptor binds native human, porcine and rat galanin with about 1 nM affinity (K_i vs. ^{125}I -galanin) and porcine galanin 1-16 at a slightly lower affinity (about 5 nM). Porcine galanin 3-29 does not bind to the receptor. The GALR1 receptor appears to couple to inhibition of adenylate cyclase, with half-maximal inhibition of forskolin-stimulated cAMP production by 1 nM galanin, and maximal inhibition occurring at about 1 μM .

Recently the rat homologue of GALR1 was cloned from the RIN14B pancreatic cell line (Burgevin *et al.*, 1995; Parker *et al.*, 1996). The pharmacologic data reported to date do not suggest substantial differences between the pharmacologic properties of the rat and human GALR1 receptors. Localization studies reveal GALR1 mRNA in rat hypothalamus, ventral hippocampus, brainstem, and spinal cord, regions consistent with roles for galanin in feeding, cognition, and pain transmission. However, GALR1 appears to be distinct from the pituitary and hippocampal receptor subtypes described above.

The indication of multiple galanin receptor subtypes within the brain underscores the importance of defining galanin receptor heterogeneity at the molecular level in order to develop specific therapeutic agents for CNS disorders. Pharmacological tools capable of distinguishing galanin receptor subtypes in tissue preparations are only beginning to appear. Several high-affinity peptide-based galanin antagonists have been developed and are proving useful in probing the functions of galanin receptors (see Bartfai *et al.*, 1993), but their peptide character precludes practical use as therapeutic agents. In light of galanin's multiple neuroendocrine roles, therapeutic agents targeting a specific disorder must be selective for the appropriate receptor subtype to minimize side effects. Thus, there is a need in the art to identify additional members of the galanin receptor protein family whose activity can be regulated to provide therapeutic effects.

SUMMARY OF THE INVENTION

It is an object of the invention to provide reagents and methods of regulating a galanin receptor-like GPCR. This and other objects of the invention are provided by one or more of the embodiments described below.

One embodiment of the invention is a galanin receptor-like GPCR polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO. 2;

the amino acid sequence shown in SEQ ID NO. 2;

amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO. 4;

the amino acid sequence shown in SEQ ID NO.4;

amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO. 5; and

5

the amino acid sequence shown in SEQ ID NO.5.

Yet another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a

10 galanin receptor-like GPCR polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO. 2;

15

the amino acid sequence shown in SEQ ID NO. 2;

amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO. 4;

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the amino acid sequence shown in SEQ ID NO.4;

amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO. 5; and

25

the amino acid sequence shown in SEQ ID NO.5.

Binding between the test compound and the galanin receptor-like GPCR polypeptide is detected. A test compound which binds to the galanin receptor-like GPCR

30 polypeptide is thereby identified as a potential agent for decreasing extracellular matrix degradation.

Another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a polynucleotide encoding a galanin receptor-like GPCR polypeptide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO. 1;

the nucleotide sequence shown in SEQ ID NO. 1;

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO. 3; and

the nucleotide sequence shown in SEQ ID NO. 3.

Binding of the test compound to the polynucleotide is detected. A test compound which binds to the polynucleotide is identified as a potential agent for decreasing extracellular matrix degradation. The agent can work by decreasing the amount of the galanin receptor-like GPCR through interacting with the galanin receptor-like GPCR mRNA.

Another embodiment of the invention is a method of screening for agents which regulate extracellular matrix degradation. A test compound is contacted with a galanin receptor-like GPCR polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO. 2;

the amino acid sequence shown in SEQ ID NO. 2;

amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO. 4;

5

the amino acid sequence shown in SEQ ID NO.4;

amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO. 5; and

10

the amino acid sequence shown in SEQ ID NO.5.

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A galanin receptor-like GPCR activity of the polypeptide is detected. A test compound which increases galanin receptor-like GPCR activity of the polypeptide relative to galanin receptor-like GPCR activity in the absence of the test compound is thereby identified as a potential agent for increasing extracellular matrix degradation.

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A test compound which decreases galanin receptor-like GPCR activity of the polypeptide relative to galanin receptor-like GPCR activity in the absence of the test compound is thereby identified as a potential agent for decreasing extracellular matrix degradation.

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Even another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a galanin receptor-like GPCR product of a polynucleotide which comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO. 1;

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the nucleotide sequence shown in SEQ ID NO. 1;

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO. 3; and

the nucleotide sequence shown in SEQ ID NO. 3.

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Binding of the test compound to the galanin receptor-like GPCR product is detected. A test compound which binds to the galanin receptor-like GPCR product is thereby identified as a potential agent for decreasing extracellular matrix degradation.

10

Still another embodiment of the invention is a method of reducing extracellular matrix degradation. A cell is contacted with a reagent which specifically binds to a polynucleotide encoding a galanin receptor-like GPCR polypeptide or the product encoded by the polynucleotide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

15

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO. 1;

the nucleotide sequence shown in SEQ ID NO. 1;

20

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO. 3; and

the nucleotide sequence shown in SEQ ID NO. 3.

25

Galanin receptor-like GPCR activity in the cell is thereby decreased.

30

The invention thus provides a human galanin receptor-like GPCR which can be used to identify galanin analogs as well as compounds which may act as galanin antagonists at the receptor site. Galanin receptor-like GPCR and fragments thereof also are useful in raising specific antibodies which can block the receptor and

effectively prevent galanin binding. Pharmaceutical compositions comprising an effective amount of a galanin receptor-like GPCR or polypeptide can be used to treat any disorder resulting from or associated with an excess of circulating galanin.

5 BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the DNA-sequence encoding a galanin receptor-like GPCR polypeptide.

10 Fig. 2 shows the amino acid sequence deduced from the DNA-sequence of Fig. 1.

Fig. 3 shows the DNA-sequence encoding a galanin receptor-like GPCR polypeptide.

15 Fig. 4 shows the amino acid sequence deduced from the DNA-sequence of Fig. 3.

Fig. 5 shows the amino acid sequence of the protein having SwissProt Accession No. P56479.

20 Fig. 6 shows the alignment of a galanin receptor-like GPCR polypeptide of Fig. 2 with the protein of Fig. 5.

Fig. 7 shows the alignment of a galanin receptor-like GPCR polypeptide of Fig. 2 with the protein of Fig. 5.

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DETAILED DESCRIPTION OF THE INVENTION

The invention relates to an isolated polynucleotide encoding a galanin receptor-like GPCR polypeptide and being selected from the group consisting of:

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- a) a polynucleotide encoding a galanin receptor-like GPCR polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 50% identical to

the amino acid sequence shown in SEQ ID NO. 2;

the amino acid sequence shown in SEQ ID NO. 2;

amino acid sequences which are at least about 50% identical to

the amino acid sequence shown in SEQ ID NO. 4;

the amino acid sequence shown in SEQ ID NO.4;

amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO. 5; and

the amino acid sequence shown in SEQ ID NO.5.

- b) polynucleotide comprising the sequence of SEQ ID NO. 1 or 3;

- c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and (b);

- d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code; and

- e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d).

Furthermore, it has been discovered by the present applicant that a galanin receptor-like GPCR, particularly a human galanin receptor-like GPCR, has been identified. This discovery provides a novel approach, through the use of heterologous expression systems, to develop subtype-selective, high-affinity, non-peptide compounds that could serve as therapeutic agents for, *inter alia*, eating disorders,

including obesity, cancer, osteoporosis, diabetes, pain, depression, ischemia, and Alzheimer's disease.

Amino acid sequences of human galanin receptor-like GPCR are shown in SEQ ID NO.2, 4 and 5. The amino acid sequence shown in SEQ ID NO.2 is 27% identical over 170 amino acids to the mouse protein identified by SwissProt Accession No. P56479 (Fig. 5) and identified as a galanin receptor (FIG. 1). Human galanin receptor-like GPCR comprises transmembrane domains from amino acids 6-23, 55-72, 77-95, and 119-136, as indicated in FIG. 1.

Polypeptides

Galanin receptor-like polypeptides according to the invention comprise at least 6, 10, 15, 25, 50, 75, 100, 125, or 130 contiguous amino acids selected from SEQ ID NO.2 or from a biologically active variant of that amino acid sequence, as defined below. A galanin receptor-like polypeptide of the invention therefore can be a portion of a galanin receptor-like GPCR, a full-length galanin receptor-like GPCR, or a fusion protein comprising all or a portion of a galanin receptor-like GPCR.

Galanin receptor-like polypeptides preferably bind galanin or a galanin analog. Activity of a GPCR such as a galanin receptor-like polypeptide can be measured using a detectably labeled galanin or galanin analog or by any of a variety of functional assays in which a biological activity mediated by the galanin receptor-like polypeptide results in an observable change in the level of some second messenger system (described in the specific examples, below). Such second messenger systems include, but are not limited to, adenylate cyclase activity, calcium mobilization, ion channel activity, inositol phospholipid hydrolysis, or guanylyl cyclase activity. Heterologous expression systems using appropriate host cells to express the galanin receptor-like polypeptide can be used to obtain the desired second messenger coupling. Receptor activity also may be assayed in an oocyte expression system.

Biologically Active Variants

5 Galanin receptor-like polypeptide variants which are biologically active, *i.e.*, retain the ability to bind galanin or a galanin analog and/or to mediate a biological activity as described above, also are galanin receptor-like polypeptides. Preferably, naturally or non-naturally occurring galanin receptor-like polypeptide variants have amino acid sequences which are at least about 50, preferably about 75, 90, 96, or 98% identical to the amino acid sequence shown in SEQ ID NO.2, 5 or 5. Percent identity between a putative galanin receptor-like polypeptide variant and an amino acid sequence of 10 SEQ ID NO.2, 4 or 5 is determined using the Blast2 alignment program (Blosun62, Expect Value 10, standard genetic codes).

15 Variations in percent identity can be due, for example, to amino acid substitutions, insertions, or deletions. Amino acid substitutions are defined as one for one amino acid replacements. They are conservative in nature when the substituted amino acid has similar structural and/or chemical properties. Examples of conservative replacements are substitution of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine.

20 Amino acid insertions or deletions are changes to or within an amino acid sequence. They typically fall in the range of about 1 to 5 amino acids. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological or immunological activity of a galanin receptor-like polypeptide can be found using computer programs well known in the art, such as DNASTAR software. 25 Whether an amino acid change results in a biologically active galanin receptor-like polypeptide can readily be determined by assaying for galanin receptor-like polypeptide activity, as described in the specific examples, below.

Fusion Proteins

Fusion proteins are useful for generating antibodies against galanin receptor-like amino acid sequences and for use in various assay systems. For example, fusion proteins can be used to identify proteins which interact with portions of a galanin receptor-like polypeptide. Protein affinity chromatography or library-based assays for protein-protein interactions, such as the yeast two-hybrid or phage display systems, can be used for this purpose. Such methods are well known in the art and also can be used as drug screens.

A galanin receptor-like polypeptide fusion protein comprises two polypeptide segments fused together by means of a peptide bond. The first polypeptide segment can comprise at least 6, 10, 15, 25, 50, 75, 100, 125, or 130 contiguous amino acids of SEQ ID NO.2, 4 or 5 or of a biologically active variant, such as those described above. The first polypeptide segment also can comprise full-length galanin receptor-like GPCR.

The second polypeptide segment can be a full-length protein or a protein fragment. Proteins commonly used in fusion protein construction include β -galactosidase, β -glucuronidase, green fluorescent protein (GFP), autofluorescent proteins, including blue fluorescent protein (BFP), glutathione-S-transferase (GST), luciferase, horseradish peroxidase (HRP), and chloramphenicol acetyltransferase (CAT). Additionally, epitope tags are used in fusion protein constructions, including histidine (His) tags, FLAG tags, influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and thioredoxin (Trx) tags. Other fusion constructions can include maltose binding protein (MBP), S-tag, Lex a DNA binding domain (DBD) fusions, GAL4 DNA binding domain fusions, and herpes simplex virus (HSV) BP16 protein fusions. A fusion protein also can be engineered to contain a cleavage site located between the galanin receptor-like polypeptide-encoding sequence and the heterologous protein sequence, so that the galanin receptor-like polypeptide can be cleaved and purified away from the heterologous moiety.

A fusion protein can be synthesized chemically, as is known in the art. Preferably, a fusion protein is produced by covalently linking two polypeptide segments or by standard procedures in the art of molecular biology. Recombinant DNA methods can be used to prepare fusion proteins, for example, by making a DNA construct which
5 comprises coding sequences selected from SEQ ID NO.1 in proper reading frame with nucleotides encoding the second polypeptide segment and expressing the DNA construct in a host cell, as is known in the art. Many kits for constructing fusion proteins are available from companies such as Promega Corporation (Madison, WI), Stratagene (La Jolla, CA), CLONTECH (Mountain View, CA), Santa Cruz
10 Biotechnology (Santa Cruz, CA), MBL International Corporation (MIC; Watertown, MA), and Quantum Biotechnologies (Montreal, Canada; 1-888-DNA-KITS).

Identification of Species Homologs

15 Species homologs of human galanin receptor-like polypeptide can be obtained using Galanin receptor-like polypeptide polynucleotides (described below) to make suitable probes or primers for screening cDNA expression libraries from other species, such as mice, monkeys, or yeast, identifying cDNAs which encode homologs of galanin receptor-like polypeptide, and expressing the cDNAs as is
20 known in the art.

Galanin Receptor-Like Polynucleotides

A galanin receptor-like polynucleotide can be single- or double-stranded and
25 comprises a coding sequence or the complement of a coding sequence for a galanin receptor-like polypeptide. The complement of coding sequences for human galanin receptor-like GPCR polypeptides are shown in SEQ ID NO.1 and 3.

Degenerate nucleotide sequences encoding human galanin receptor-like polypeptides,
30 as well as homologous nucleotide sequences which are at least about 50, preferably about 75, 90, 96, or 98% identical to the complement of a nucleotide sequence shown

in SEQ ID NO.1 and 3 also are galanin receptor-like polynucleotides. Percent sequence identity between the sequences of two polynucleotides is determined using computer programs such as ALIGN which employ the FASTA algorithm, using an affine gap search with a gap open penalty of -12 and a gap extension penalty of -2.

5 Complementary DNA (cDNA) molecules, species homologs, and variants of galanin receptor-like polynucleotides which encode biologically active Galanin receptor-like polypeptides also are galanin receptor-like polynucleotides.

Identification of Polynucleotide Variants and Homologs

10 Variants and homologs of the galanin receptor-like polynucleotides described above also are galanin receptor-like polynucleotides. Typically, homologous galanin receptor-like polynucleotide sequences can be identified by hybridization of candidate polynucleotides to known galanin receptor-like polynucleotides under

15 stringent conditions, as is known in the art. For example, using the following wash conditions--2X SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0), 0.1% SDS, room temperature twice, 30 minutes each; then 2X SSC, 0.1% SDS, 50°C once, 30 minutes; then 2X SSC, room temperature twice, 10 minutes each--homologous

20 sequences can be identified which contain at most about 25-30% basepair mismatches. More preferably, homologous nucleic acid strands contain 15-25% basepair mismatches, even more preferably 5-15% basepair mismatches.

Species homologs of the galanin receptor-like polynucleotides disclosed herein also can be identified by making suitable probes or primers and screening cDNA

25 expression libraries from other species, such as mice, monkeys, or yeast. Human variants of galanin receptor-like polynucleotides can be identified, for example, by screening human cDNA expression libraries. It is well known that the T_m of a double-stranded DNA decreases by 1-1.5 °C with every 1% decrease in homology (Bonner *et al.*, *J. Mol. Biol.* 81, 123 (1973). Variants of human galanin receptor-like

30 polynucleotides or galanin receptor-like polynucleotides of other species can therefore be identified by hybridizing a putative homologous galanin receptor-like

polynucleotide with a polynucleotide having a nucleotide sequence of SEQ ID NO.1 or 3 or the complement thereof to form a test hybrid. The melting temperature of the test hybrid is compared with the melting temperature of a hybrid comprising polynucleotides having perfectly complementary nucleotide sequences, and the number or percent of basepair mismatches within the test hybrid is calculated.

Nucleotide sequences which hybridize to galanin receptor-like polynucleotides or their complements following stringent hybridization and/or wash conditions are also galanin receptor-like polynucleotides. Stringent wash conditions are well known and understood in the art and are disclosed, for example, in Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, 2d ed., 1989, at pages 9.50-9.51.

Typically, for stringent hybridization conditions a combination of temperature and salt concentration should be chosen that is approximately 12-20°C below the calculated T_m of the hybrid under study. The T_m of a hybrid between a galanin receptor-like polynucleotide having a nucleotide sequence shown in SEQ ID NO.1 or 3 or the complement thereof and a polynucleotide sequence which is at least about 50, preferably about 75, 90, 96, or 98% identical to one of those nucleotide sequences can be calculated, for example, using the equation of Bolton and McCarthy, *Proc. Natl. Acad. Sci. U.S.A.* 48, 1390 (1962):

$$T_m = 81.5^{\circ}\text{C} - 16.6(\log_{10}[\text{Na}^+]) + 0.41(\%G + C) - 0.63(\%\text{formamide}) - 600/l,$$

where l = the length of the hybrid in basepairs.

Stringent wash conditions include, for example, 4X SSC at 65°C, or 50% formamide, 4X SSC at 42°C, or 0.5X SSC, 0.1% SDS at 65°C. Highly stringent wash conditions include, for example, 0.2X SSC at 65°C.

Preparation of Polynucleotides

A naturally occurring galanin receptor-like polynucleotide can be isolated free of other cellular components such as membrane components, proteins, and lipids. Polynucleotides can be made by a cell and isolated using standard nucleic acid purification techniques, or synthesized using an amplification technique, such as the polymerase chain reaction (PCR), or by using an automatic synthesizer. Methods for isolating polynucleotides are routine and are known in the art. Any such technique for obtaining a polynucleotide can be used to obtain isolated galanin receptor-like polynucleotides. For example, restriction enzymes and probes can be used to isolate polynucleotide fragments which comprises galanin receptor-like nucleotide sequences. Isolated polynucleotides are in preparations which are free or at least 70, 80, or 90% free of other molecules.

Galanin receptor-like cDNA molecules can be made with standard molecular biology techniques, using galanin receptor-like mRNA as a template. Galanin receptor-like cDNA molecules can thereafter be replicated using molecular biology techniques known in the art and disclosed in manuals such as Sambrook *et al.* (1989). An amplification technique, such as PCR, can be used to obtain additional copies of polynucleotides of the invention, using either human genomic DNA or cDNA as a template.

Alternatively, synthetic chemistry techniques can be used to synthesize galanin receptor-like polynucleotides. The degeneracy of the genetic code allows alternate nucleotide sequences to be synthesized which will encode a galanin receptor-like polypeptide having, for example, an amino acid sequence shown in SEQ ID NO.2, 4 or 5 or a biologically active variant thereof.

Extending Polynucleotides

Various PCR-based methods can be used to extend nucleic acid sequences encoding human galanin receptor-like GPCR to detect upstream sequences such as promoters and regulatory elements. For example, restriction-site PCR uses universal primers to

retrieve unknown sequence adjacent to a known locus (Sarkar, *PCR Methods Applic.* 2, 318-322, 1993). Genomic DNA is first amplified in the presence of a primer to a linker sequence and a primer specific to the known region. The amplified sequences are then subjected to a second round of PCR with the same linker primer and another
5 specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR also can be used to amplify or extend sequences using divergent primers
10 based on a known region (Triglia *et al.*, *Nucleic Acids Res.* 16, 8186, 1988). Primers can be designed using commercially available software, such as OLIGO 4.06 Primer Analysis software (National Biosciences Inc., Plymouth, Minn.), to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the
15 target sequence at temperatures about 68-72°C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Another method which can be used is capture PCR, which involves PCR
20 amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA (Lagerstrom *et al.*, *PCR Methods Applic.* 1, 111-119, 1991). In this method, multiple restriction enzyme digestions and ligations also can be used to place an engineered double-stranded sequence into an unknown fragment of the DNA molecule before performing PCR.

25 Another method which can be used to retrieve unknown sequences is that of Parker *et al.*, *Nucleic Acids Res.* 19, 3055-3060, 1991). Additionally, PCR, nested primers, and PROMOTERFINDER libraries (CLONTECH, Palo Alto, Calif.) can be used to walk genomic DNA (CLONTECH, Palo Alto, Calif.). This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Randomly-primed libraries are preferable, in that they will contain more sequences which contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries can be useful for extension of sequence into 5' non-transcribed regulatory regions.

Commercially available capillary electrophoresis systems can be used to analyze the size or confirm the nucleotide sequence of PCR or sequencing products. For example, capillary sequencing can employ flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) which are laser activated, and detection of the emitted wavelengths by a charge coupled device camera. Output/light intensity can be converted to electrical signal using appropriate software (e.g. GENOTYPER and Sequence NAVIGATOR, Perkin Elmer), and the entire process from loading of samples to computer analysis and electronic data display can be computer controlled. Capillary electrophoresis is especially preferable for the sequencing of small pieces of DNA which might be present in limited amounts in a particular sample.

Obtaining Polypeptides

Galanin receptor-like polypeptides can be obtained, for example, by purification from human cells, by expression of galanin receptor-like polynucleotides, or by direct chemical synthesis.

Protein Purification

Galanin receptor-like polypeptides can be purified from any human cell which expresses the receptor, including those which have been transfected with expression constructs which express galanin receptor-like polypeptides. Kidney tumors, fetal lung, testis, and B cells are particularly useful sources of human galanin receptor-like

polypeptides. A purified galanin receptor-like polypeptide is separated from other compounds which normally associate with the galanin receptor-like polypeptide in the cell, such as certain proteins, carbohydrates, or lipids, using methods well-known in the art. Such methods include, but are not limited to, size exclusion chromatography, ammonium sulfate fractionation, ion exchange chromatography, affinity chromatography, and preparative gel electrophoresis.

Galanin receptor-like polypeptide is conveniently isolated as a complex with its associated G protein. A variety of galanin analogs are available and can be used as ligands for receptor binding. See the specific examples, below. A preparation of purified galanin receptor-like polypeptides is at least 80% pure; preferably, the preparations are 90%, 95%, or 99% pure. Purity of the preparations can be assessed by any means known in the art, such as SDS-polyacrylamide gel electrophoresis.

Expression of Galanin Receptor-Like Polynucleotides

To express a galanin receptor-like polypeptide, a galanin receptor-like polynucleotide can be inserted into an expression vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art can be used to construct expression vectors containing sequences encoding galanin receptor-like polypeptides and appropriate transcriptional and translational control elements. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Such techniques are described, for example, in Sambrook *et al.* (1989) and in Ausubel *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, N.Y., 1989.

A variety of expression vector/host systems can be utilized to contain and express sequences encoding a galanin receptor-like polypeptide. These include, but are not limited to, microorganisms, such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with

yeast expression vectors, insect cell systems infected with virus expression vectors (e.g., baculovirus), plant cell systems transformed with virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids), or animal cell systems.

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The control elements or regulatory sequences are those non-translated regions of the vector -- enhancers, promoters, 5' and 3' untranslated regions -- which interact with host cellular proteins to carry out transcription and translation. Such elements can vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, can be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the BLUESCRIPT phagemid (Stratagene, LaJolla, Calif.) or pSPORT1 plasmid (Life Technologies) and the like can be used. The baculovirus polyhedrin promoter can be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (e.g., heat shock, RUBISCO, and storage protein genes) or from plant viruses (e.g., viral promoters or leader sequences) can be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of a nucleotide sequence encoding a galanin receptor-like polypeptide, vectors based on SV40 or EBV can be used with an appropriate selectable marker.

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Bacterial and Yeast Expression Systems

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In bacterial systems, a number of expression vectors can be selected depending upon the use intended for the galanin receptor-like polypeptide. For example, when a large quantity of a galanin receptor-like polypeptide is needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified can be used. Such vectors include, but are not limited to, multifunctional *E. coli* cloning and expression vectors such as BLUESCRIPT (Stratagene). In a BLUESCRIPT vector, a sequence encoding the galanin receptor-

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like polypeptide can be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of β -galactosidase so that a hybrid protein is produced. pIN vectors (Van Heeke & Schuster, *J. Biol. Chem.* 264, 5503-5509, 1989) or pGEX vectors (Promega, Madison, Wis.) also can be used to express
5 foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems can be designed to include heparin, thrombin, or factor Xa protease cleavage sites so that the cloned polypeptide of
10 interest can be released from the GST moiety at will.

In the yeast *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH can be used. For reviews, see Ausubel *et al.* (1989) and Grant *et al.*, *Methods Enzymol.* 153, 516-
15 544, 1987.

Plant and Insect Expression Systems

If plant expression vectors are used, the expression of sequences encoding galanin receptor-like polypeptides can be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV can be used alone or in combination with the omega leader sequence from TMV (Takamatsu *EMBO J.* 6, 307-311, 1987). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters can be used (Coruzzi *et al.*, *EMBO J.* 3, 1671-1680, 1984; Broglie *et al.*, *Science* 224, 838-843, 1984; Winter *et al.*, *Results Probl. Cell Differ.* 17, 85-105, 1991). These constructs can be introduced into plant cells by direct DNA transformation or by pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (*e.g.*, Hobbs or Murray, in MCGRAW HILL YEARBOOK OF SCIENCE AND TECHNOLOGY, McGraw Hill, New York, N.Y., pp. 191-196, 1992).

An insect system also can be used to express a galanin receptor-like polypeptide. For example, in one such system *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. Sequences encoding galanin receptor-like polypeptides can be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of galanin receptor-like polypeptides will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses can then be used to infect *S. frugiperda* cells or *Trichoplusia* larvae in which galanin receptor-like polypeptides can be expressed (Engelhard *et al.*, *Proc. Nat. Acad. Sci.* 91, 3224-3227, 1994).

Mammalian Expression Systems

A number of viral-based expression systems can be used to express galanin receptor-like polypeptides in mammalian host cells. For example, if an adenovirus is used as

- an expression vector, sequences encoding galanin receptor-like polypeptides can be ligated into an adenovirus transcription/translation complex comprising the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome can be used to obtain a viable virus which is capable of expressing a galanin receptor-like polypeptide in infected host cells (Logan & Shenk, *Proc. Natl. Acad. Sci.* 81, 3655-3659, 1984). If desired, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, can be used to increase expression in mammalian host cells.
- Human artificial chromosomes (HACs) also can be used to deliver larger fragments of DNA than can be contained and expressed in a plasmid. HACs of 6M to 10M are constructed and delivered to cells via conventional delivery methods (e.g., liposomes, polycationic amino polymers, or vesicles).
- Specific initiation signals also can be used to achieve more efficient translation of sequences encoding galanin receptor-like polypeptides. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding a galanin receptor-like polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals (including the ATG initiation codon) should be provided. The initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used (see Scharf *et al.*, *Results Probl. Cell Differ.* 20, 125-162, 1994).

Host Cells

A host cell strain can be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed galanin receptor-like polypeptide in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the polypeptide also can be used to facilitate correct insertion, folding and/or function. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC; 10801 University Boulevard, Manassas, VA 20110-2209) and can be chosen to ensure the correct modification and processing of the foreign protein.

Stable expression is preferred for long-term, high-yield production of recombinant proteins. For example, cell lines which stably express galanin receptor-like polypeptides can be transformed using expression vectors which can contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells can be allowed to grow for 1-2 days in an enriched medium before they are switched to a selective medium. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced galanin receptor-like sequences. Resistant clones of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell type. See, for example, ANIMAL CELL CULTURE, R.I. Freshney, ed., 1986.

Any number of selection systems can be used to recover transformed cell lines.

These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler *et al.*, *Cell* 11, 223-32, 1977) and adenine phosphoribosyltransferase (Lowy

et al., *Cell* 22, 817-23, 1980) genes which can be employed in *tk* or *aprt* cells, respectively. Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate (Wigler et al., *Proc. Natl. Acad. Sci.* 77, 3567-70, 1980) *npt* confers resistance to the aminoglycosides, neomycin and G-418 (Colbere-Garapin et al., *J. Mol. Biol.* 150, 1-14, 1981), and *als* and *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murray, 1992, *supra*). Additional selectable genes have been described. For example, *trpB* allows cells to utilize indole in place of tryptophan, or *hisD*, which allows cells to utilize histinol in place of histidine (Hartman & Mulligan, *Proc. Natl. Acad. Sci.* 85, 8047-51, 1988). Visible markers such as anthocyanins, β -glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, can be used to identify transformants and to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes et al., *Methods Mol. Biol.* 55, 121-131, 1995).

Detecting Polypeptide Expression

Although the presence of marker gene expression suggests that the galanin receptor-like polynucleotide is also present, its presence and expression may need to be confirmed. For example, if a sequence encoding a galanin receptor-like polypeptide is inserted within a marker gene sequence, transformed cells containing sequences which encode a galanin receptor-like polypeptide can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding a galanin receptor-like polypeptide under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the galanin receptor-like polynucleotide.

Alternatively, host cells which contain a galanin receptor-like polynucleotide and which express a galanin receptor-like polypeptide can be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not

limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques which include membrane, solution, or chip-based technologies for the detection and/or quantification of nucleic acid or protein. For example, the presence of a polynucleotide sequence encoding a galanin receptor-like polypeptide can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes or fragments or fragments of polynucleotides encoding a galanin receptor-like polypeptide. Nucleic acid amplification-based assays involve the use of oligonucleotides selected from sequences encoding a galanin receptor-like polypeptide to detect transformants which contain a galanin receptor-like polynucleotide.

A variety of protocols for detecting and measuring the expression of a galanin receptor-like polypeptide, using either polyclonal or monoclonal antibodies specific for the polypeptide, are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay using monoclonal antibodies reactive to two non-interfering epitopes on a galanin receptor-like polypeptide can be used, or a competitive binding assay can be employed. These and other assays are described in Hampton *et al.*, SEROLOGICAL METHODS: A LABORATORY MANUAL, APS Press, St. Paul, Minn., 1990) and Maddox *et al.*, *J. Exp. Med.* 158, 1211-1216, 1983).

A wide variety of labels and conjugation techniques are known by those skilled in the art and can be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding galanin receptor-like polypeptides include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, sequences encoding a galanin receptor-like polypeptide can be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and can be used to synthesize RNA probes *in vitro* by addition of labeled nucleotides and an appropriate RNA polymerase such as T7,

T3, or SP6. These procedures can be conducted using a variety of commercially available kits (Amersham Pharmacia Biotech, Promega, and US Biochemical). Suitable reporter molecules or labels which can be used for ease of detection include radionuclides, enzymes, and fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Expression and Purification of Polypeptides

Host cells transformed with nucleotide sequences encoding a galanin receptor-like polypeptide can be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The polypeptide produced by a transformed cell can be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode galanin receptor-like polypeptides can be designed to contain signal sequences which direct secretion of soluble galanin receptor-like polypeptides through a prokaryotic or eukaryotic cell membrane or which direct the membrane insertion of membrane-bound galanin receptor-like polypeptide.

As discussed above, other constructions can be used to join a sequence encoding a galanin receptor-like polypeptide to a nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, Wash.). Inclusion of cleavable linker sequences such as those specific for Factor XA or enterokinase (Invitrogen, San Diego, CA) between the purification domain and the galanin receptor-like polypeptide also can be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing a galanin receptor-like polypeptide and 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate

purification by IMAC (immobilized metal ion affinity chromatography, as described in Porath *et al.*, *Prot. Exp. Purif.* 3, 263-281, 1992), while the enterokinase cleavage site provides a means for purifying the galanin receptor-like polypeptide from the fusion protein. Vectors which contain fusion proteins are disclosed in Kroll *et al.*,
5 *DNA Cell Biol.* 12, 441-453, 1993.

Chemical Synthesis

Sequences encoding a galanin receptor-like polypeptide can be synthesized, in whole
10 or in part, using chemical methods well known in the art (see Caruthers *et al.*, *Nucl. Acids Res. Symp. Ser.* 215-223, 1980; Horn *et al.* *Nucl. Acids Res. Symp. Ser.* 225-232, 1980). Alternatively, a galanin receptor-like polypeptide itself can be produced using chemical methods to synthesize its amino acid sequence, such as by direct peptide synthesis using solid-phase techniques (Merrifield, *J. Am. Chem. Soc.* 85,
15 2149-2154, 1963; Roberge *et al.*, *Science* 269, 202-204, 1995). Protein synthesis can be performed using manual techniques or by automation. Automated synthesis can be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Optionally, fragments of galanin receptor-like polypeptides can be separately synthesized and combined using chemical methods to produce a full-
20 length molecule.

The newly synthesized peptide can be substantially purified by preparative high performance liquid chromatography (*e.g.*, Creighton, *PROTEINS: STRUCTURES AND MOLECULAR PRINCIPLES*, WH Freeman and Co., New York, N.Y., 1983). The
25 composition of a synthetic galanin receptor-like polypeptide can be confirmed by amino acid analysis or sequencing (*e.g.*, the Edman degradation procedure; *see* Creighton, *supra*). Additionally, any portion of the amino acid sequence of the galanin receptor-like polypeptide can be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins to produce a
30 variant polypeptide or a fusion protein.

Production of Altered Polypeptides

As will be understood by those of skill in the art, it may be advantageous to produce galanin receptor-like polypeptide-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce an RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

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The nucleotide sequences disclosed herein can be engineered using methods generally known in the art to alter galanin receptor-like polypeptide-encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the polypeptide or mRNA product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides can be used to engineer the nucleotide sequences. For example, site-directed mutagenesis can be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, introduce mutations, and so forth.

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Antibodies

Any type of antibody known in the art can be generated to bind specifically to an epitope of a galanin receptor-like polypeptide. "Antibody" as used herein includes intact immunoglobulin molecules, as well as fragments thereof, such as Fab, F(ab')₂, and Fv, which are capable of binding an epitope of a galanin receptor-like polypeptide. Typically, at least 6, 8, 10, or 12 contiguous amino acids are required to form an epitope. However, epitopes which involve non-contiguous amino acids may require more, e.g., at least 15, 25, or 50 amino acids.

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An antibody which specifically binds to an epitope of a galanin receptor-like polypeptide can be used therapeutically, as well as in immunochemical assays, such as Western blots, ELISAs, radioimmunoassays, immunohistochemical assays, immunoprecipitations, or other immunochemical assays known in the art. Various immunoassays can be used to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays are well known in the art. Such immunoassays typically involve the measurement of complex formation between an immunogen and an antibody which specifically binds to the immunogen.

Typically, an antibody which specifically binds to a galanin receptor-like polypeptide provides a detection signal at least 5-, 10-, or 20-fold higher than a detection signal provided with other proteins when used in an immunochemical assay. Preferably, antibodies which specifically bind to galanin receptor-like polypeptides do not detect other proteins in immunochemical assays and can immunoprecipitate a galanin receptor-like polypeptide from solution.

Galanin receptor-like polypeptides can be used to immunize a mammal, such as a mouse, rat, rabbit, guinea pig, monkey, or human, to produce polyclonal antibodies. If desired, a galanin receptor-like polypeptide can be conjugated to a carrier protein, such as bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin. Depending on the host species, various adjuvants can be used to increase the immunological response. Such adjuvants include, but are not limited to, Freund's adjuvant, mineral gels (e.g., aluminum hydroxide), and surface active substances (e.g. lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol). Among adjuvants used in humans, BCG (*bacilli Calmette-Guerin*) and *Corynebacterium parvum* are especially useful.

Monoclonal antibodies which specifically bind to a galanin receptor-like polypeptide can be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These techniques include, but are not

limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (Kohler *et al.*, *Nature* 256, 495-497, 1985; Kozbor *et al.*, *J. Immunol. Methods* 81, 31-42, 1985; Cote *et al.*, *Proc. Natl. Acad. Sci.* 80, 2026-2030, 1983; Cole *et al.*, *Mol. Cell Biol.* 62, 109-120, 1984).

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In addition, techniques developed for the production of "chimeric antibodies," the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used (Morrison *et al.*, *Proc. Natl. Acad. Sci.* 81, 6851-6855, 1984; Neuberger *et al.*, *Nature* 312, 604-608, 1984; Takeda *et al.*, *Nature* 314, 452-454, 1985). Monoclonal and other antibodies also can be "humanized" to prevent a patient from mounting an immune response against the antibody when it is used therapeutically. Such antibodies may be sufficiently similar in sequence to human antibodies to be used directly in therapy or may require alteration of a few key residues. Sequence differences between rodent antibodies and human sequences can be minimized by replacing residues which differ from those in the human sequences by site directed mutagenesis of individual residues or by grafting of entire complementarity determining regions. Alternatively, humanized antibodies can be produced using recombinant methods, as described in GB2188638B. Antibodies which specifically bind to a galanin receptor-like polypeptide can contain antigen binding sites which are either partially or fully humanized, as disclosed in U.S. 5,565,332.

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Alternatively, techniques described for the production of single chain antibodies can be adapted using methods known in the art to produce single chain antibodies which specifically bind to galanin receptor-like polypeptides. Antibodies with related specificity, but of distinct idiotypic composition, can be generated by chain shuffling from random combinatorial immunoglobulin libraries (Burton, *Proc. Natl. Acad. Sci.* 88, 11120-23, 1991).

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Single-chain antibodies also can be constructed using a DNA amplification method, such as PCR, using hybridoma cDNA as a template (Thirion *et al.*, 1996, *Eur. J.*

Cancer Prev. 5, 507-11). Single-chain antibodies can be mono- or bispecific, and can be bivalent or tetravalent. Construction of tetravalent, bispecific single-chain antibodies is taught, for example, in Coloma & Morrison, 1997, *Nat. Biotechnol.* 15, 159-63. Construction of bivalent, bispecific single-chain antibodies is taught in
5 Mallender & Voss, 1994, *J. Biol. Chem.* 269, 199-206.

A nucleotide sequence encoding a single-chain antibody can be constructed using manual or automated nucleotide synthesis, cloned into an expression construct using standard recombinant DNA methods, and introduced into a cell to express the coding
10 sequence, as described below. Alternatively, single-chain antibodies can be produced directly using, for example, filamentous phage technology (Verhaar *et al.*, 1995, *Int. J. Cancer* 61, 497-501; Nicholls *et al.*, 1993, *J. Immunol. Meth.* 165, 81-91).

15 Antibodies which specifically bind to galanin receptor-like polypeptides also can be produced by inducing *in vivo* production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (Orlandi *et al.*, *Proc. Natl. Acad. Sci.* 86, 3833-3837, 1989; Winter *et al.*, *Nature* 349, 293-299, 1991).

20 Other types of antibodies can be constructed and used therapeutically in methods of the invention. For example, chimeric antibodies can be constructed as disclosed in WO 93/03151. Binding proteins which are derived from immunoglobulins and which are multivalent and multispecific, such as the "diabodies" described in WO
25 94/13804, also can be prepared.

Antibodies according to the invention can be purified by methods well known in the art. For example, antibodies can be affinity purified by passage over a column to which a galanin receptor-like polypeptide is bound. The bound antibodies can then
30 be eluted from the column using a buffer with a high salt concentration.

Antisense Oligonucleotides

Antisense oligonucleotides are nucleotide sequences which are complementary to a specific DNA or RNA sequence. Once introduced into a cell, the complementary
5 nucleotides combine with natural sequences produced by the cell to form complexes and block either transcription or translation. Preferably, an antisense oligonucleotide is at least 11 nucleotides in length, but can be at least 12, 15, 20, 25, 30, 35, 40, 45, or 50 or more nucleotides long. Longer sequences also can be used. Antisense oligonucleotide molecules can be provided in a DNA construct and introduced into a
10 cell as described above to decrease the level of galanin receptor-like GPCR gene products in the cell.

Antisense oligonucleotides can be deoxyribonucleotides, ribonucleotides, or a combination of both. Oligonucleotides can be synthesized manually or by an
15 automated synthesizer, by covalently linking the 5' end of one nucleotide with the 3' end of another nucleotide with non-phosphodiester internucleotide linkages such as alkylphosphonates, phosphorothioates, phosphorodithioates, alkylphosphonothioates, alkylphosphonates, phosphoramidates, phosphate esters, carbamates, acetamidate, carboxymethyl esters, carbonates, and phosphate triesters. See Brown, *Meth. Mol. Biol.* 20, 1-8, 1994; Sonveaux, *Meth. Mol. Biol.* 26, 1-72, 1994; Uhlmann *et al.*,
20 *Chem. Rev.* 90, 543-583, 1990.

Modifications of galanin receptor-like GPCR gene expression can be obtained by designing antisense oligonucleotides which will form duplexes to the control, 5', or
25 regulatory regions of the galanin receptor-like GPCR gene. Oligonucleotides derived from the transcription initiation site, *e.g.*, between positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases,
30 transcription factors, or chaperons. Therapeutic advances using triplex DNA have been described in the literature (*e.g.*, Gee *et al.*, in Huber & Cart, MOLECULAR AND

IMMUNOLOGIC APPROACHES, Futura Publishing Co., Mt. Kisco, N.Y., 1994). An antisense oligonucleotide also can be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

5 Precise complementarity is not required for successful complex formation between
an antisense oligonucleotide and the complementary sequence of a galanin receptor-
like polynucleotide. Antisense oligonucleotides which comprise, for example, 2, 3,
4, or 5 or more stretches of contiguous nucleotides which are precisely
10 complementary to a galanin receptor-like polynucleotide, each separated by a stretch
of contiguous nucleotides which are not complementary to adjacent galanin receptor-
like GPCR nucleotides, can provide sufficient targeting specificity for galanin
receptor-like GPCR mRNA. Preferably, each stretch of complementary contiguous
15 nucleotides is at least 4, 5, 6, 7, or 8 or more nucleotides in length. Non-
complementary intervening sequences are preferably 1, 2, 3, or 4 nucleotides in
length. One skilled in the art can easily use the calculated melting point of an
antisense-sense pair to determine the degree of mismatching which will be tolerated
between a particular antisense oligonucleotide and a particular galanin receptor-like
polynucleotide sequence.

20 Antisense oligonucleotides can be modified without affecting their ability to
hybridize to a galanin receptor-like polynucleotide. These modifications can be
internal or at one or both ends of the antisense molecule. For example,
internucleoside phosphate linkages can be modified by adding cholesteryl or diamine
25 moieties with varying numbers of carbon residues between the amino groups and
terminal ribose. Modified bases and/or sugars, such as arabinose instead of ribose, or
a 3', 5'-substituted oligonucleotide in which the 3' hydroxyl group or the 5' phosphate
group are substituted, also can be employed in a modified antisense oligonucleotide.
These modified oligonucleotides can be prepared by methods well known in the art.
See, e.g., Agrawal *et al.*, *Trends Biotechnol.* 10, 152-158, 1992; Uhlmann *et al.*,
30 *Chem. Rev.* 90, 543-584, 1990; Uhlmann *et al.*, *Tetrahedron. Lett.* 215, 3539-3542,
1987.

Ribozymes

Ribozymes are RNA molecules with catalytic activity. See, e.g., Cech, *Science* 236, 1532-1539; 1987; Cech, *Ann. Rev. Biochem.* 59, 543-568; 1990, Cech, *Curr. Opin. Struct. Biol.* 2, 605-609; 1992, Couture & Stinchcomb, *Trends Genet.* 12, 510-515, 1996. Ribozymes can be used to inhibit gene function by cleaving an RNA sequence, as is known in the art (e.g., Haseloff *et al.*, U.S. Patent 5,641,673). The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Examples include engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of specific nucleotide sequences.

The coding sequence of a galanin receptor-like polynucleotide can be used to generate ribozymes which will specifically bind to mRNA transcribed from the galanin receptor-like polynucleotide. Methods of designing and constructing ribozymes which can cleave other RNA molecules in trans in a highly sequence specific manner have been developed and described in the art (see Haseloff *et al.* *Nature* 334, 585-591, 1988). For example, the cleavage activity of ribozymes can be targeted to specific RNAs by engineering a discrete "hybridization" region into the ribozyme. The hybridization region contains a sequence complementary to the target RNA and thus specifically hybridizes with the target (see, for example, Gerlach *et al.*, EP 321,201).

Specific ribozyme cleavage sites within a galanin receptor-like GPCR RNA target can be identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target RNA containing the cleavage site can be evaluated for secondary structural features which may render the target inoperable. Suitability of candidate galanin

receptor-like GPCR RNA targets also can be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays. The nucleotide sequences shown in SEQ ID NO.1 and its complement provide sources of suitable hybridization region sequences. Longer complementary sequences can be used to increase the affinity of the hybridization sequence for the target. The hybridizing and cleavage regions of the ribozyme can be integrally related such that upon hybridizing to the target RNA through the complementary regions, the catalytic region of the ribozyme can cleave the target.

Ribozymes can be introduced into cells as part of a DNA construct. Mechanical methods, such as microinjection, liposome-mediated transfection, electroporation, or calcium phosphate precipitation, can be used to introduce a ribozyme-containing DNA construct into cells in which it is desired to decrease galanin receptor-like GPCR expression. Alternatively, if it is desired that the cells stably retain the DNA construct, the construct can be supplied on a plasmid and maintained as a separate element or integrated into the genome of the cells, as is known in the art. A ribozyme-encoding DNA construct can include transcriptional regulatory elements, such as a promoter element, an enhancer or UAS element, and a transcriptional terminator signal, for controlling transcription of ribozymes in the cells.

As taught in Haseloff *et al.*, U.S. Patent 5,641,673, ribozymes can be engineered so that ribozyme expression will occur in response to factors which induce expression of a target gene. Ribozymes also can be engineered to provide an additional level of regulation, so that destruction of mRNA occurs only when both a ribozyme and a target gene are induced in the cells.

Screening Methods

The invention provides methods for identifying modulators, *i.e.*, candidate or test compounds which bind to galanin receptor-like polypeptides or polynucleotides and/or have a stimulatory or inhibitory effect on, for example, expression or activity

of the galanin receptor-like polypeptide or polynucleotide, so as to increase or decrease signal transduction mediated by the receptor.

5 The invention provides assays for screening test compounds which bind to or modulate the activity of a galanin receptor-like polypeptide or a galanin receptor-like polynucleotide. A test compound preferably binds to a galanin receptor-like polypeptide or polynucleotide. More preferably, a test compound decreases or increases a galanin receptor-like GPCR activity of a galanin receptor-like polypeptide or expression of a galanin receptor-like polynucleotide by at least about 10, 10 preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the test compound.

Test Compounds

15 Test compounds can be pharmacologic agents already known in the art or can be compounds previously unknown to have any pharmacological activity. The compounds can be naturally occurring or designed in the laboratory. They can be isolated from microorganisms, animals, or plants, and can be produced recombinantly, or synthesized by chemical methods known in the art. If desired, test 20 compounds can be obtained using any of the numerous combinatorial library methods known in the art, including but not limited to, biological libraries, spatially addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the "one-bead one-compound" library method, and synthetic library methods using affinity chromatography selection. The biological 25 library approach is limited to polypeptide libraries, while the other four approaches are applicable to polypeptide, non-peptide oligomer, or small molecule libraries of compounds. See Lam, *Anticancer Drug Des.* 12, 145, 1997.

30 Methods for the synthesis of molecular libraries are well known in the art (see, for example, DeWitt *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 90, 6909, 1993; Erb *et al.* *Proc. Natl. Acad. Sci. U.S.A.* 91, 11422, 1994; Zuckermann *et al.*, *J. Med. Chem.* 37, 2678,

1994; Cho *et al.*, *Science* 261, 1303, 1993; Carell *et al.*, *Angew. Chem. Int. Ed. Engl.* 33, 2059, 1994; Carell *et al.*, *Angew. Chem. Int. Ed. Engl.* 33, 2061; Gallop *et al.*, *J. Med. Chem.* 37, 1233, 1994). Libraries of compounds can be presented in solution (see, e.g., Houghten, *BioTechniques* 13, 412-421, 1992), or on beads (Lam, *Nature* 354, 82-84, 1991), chips (Fodor, *Nature* 364, 555-556, 1993), bacteria or spores (Ladner, U.S. Patent 5,223,409), plasmids (Cull *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 89, 1865-1869, 1992), or phage (Scott & Smith, *Science* 249, 386-390, 1990; Devlin, *Science* 249, 404-406, 1990); Cwirla *et al.*, *Proc. Natl. Acad. Sci.* 97, 6378-6382, 1990; Felici, *J. Mol. Biol.* 222, 301-310, 1991; and Ladner, U.S. Patent 5,223,409).

High Throughput Screening

Test compounds can be screened for the ability to bind to galanin receptor-like polypeptides or polynucleotides or to affect galanin receptor-like GPCR activity or galanin receptor-like GPCR gene expression using high throughput screening. Using high throughput screening, many discrete compounds can be tested in parallel so that large numbers of test compounds can be quickly screened. The most widely established techniques utilize 96-well microtiter plates. The wells of the microtiter plates typically require assay volumes that range from 50 to 500 μ l. In addition to the plates, many instruments, materials, pipettors, robotics, plate washers, and plate readers are commercially available to fit the 96-well format.

Alternatively, "free format assays," or assays that have no physical barrier between samples, can be used. For example, an assay using pigment cells (melanocytes) in a simple homogeneous assay for combinatorial peptide libraries is described by Jayawickreme *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 19, 1614-18 (1994). The cells are placed under agarose in petri dishes, then beads that carry combinatorial compounds are placed on the surface of the agarose. The combinatorial compounds are partially released the compounds from the beads. Active compounds can be visualized as

dark pigment areas because, as the compounds diffuse locally into the gel matrix, the active compounds cause the cells to change colors.

5 Another example of a free format assay is described by Chelsky, "Strategies for Screening Combinatorial Libraries: Novel and Traditional Approaches," reported at the First Annual Conference of The Society for Biomolecular Screening in Philadelphia, Pa. (Nov. 7-10, 1995). Chelsky placed a simple homogenous enzyme assay for carbonic anhydrase inside an agarose gel such that the enzyme in the gel would cause a color change throughout the gel. Thereafter, beads carrying
10 combinatorial compounds via a photolinker were placed inside the gel and the compounds were partially released by UV-light. Compounds that inhibited the enzyme were observed as local zones of inhibition having less color change.

15 Yet another example is described by Salmon *et al.*, *Molecular Diversity* 2, 57-63 (1996). In this example, combinatorial libraries were screened for compounds that had cytotoxic effects on cancer cells growing in agar.

20 Another high throughput screening method is described in Beutel *et al.*, U.S. Patent 5,976,813. In this method, test samples are placed in a porous matrix. One or more assay components are then placed within, on top of, or at the bottom of a matrix such as a gel, a plastic sheet, a filter, or other form of easily manipulated solid support. When samples are introduced to the porous matrix they diffuse sufficiently slowly, such that the assays can be performed without the test samples running together.

25 Binding Assays

30 For binding assays, the test compound is preferably a small molecule which binds to and occupies the active site of the galanin receptor-like polypeptide, thereby making the ligand binding site inaccessible to substrate such that normal biological activity is prevented. Examples of such small molecules include, but are not limited to, small peptides or peptide-like molecules. Potential ligands which bind to a polypeptide of

the invention include, but are not limited to, the natural ligands of known galanin receptor-like GPCRs and analogues or derivatives thereof.

5 In binding assays, either the test compound or the galanin receptor-like polypeptide can comprise a detectable label, such as a fluorescent, radioisotopic, chemiluminescent, or enzymatic label, such as horseradish peroxidase, alkaline phosphatase, or luciferase. Detection of a test compound which is bound to the galanin receptor-like polypeptide can then be accomplished, for example, by direct counting of radioemmission, by scintillation counting, or by determining conversion of an
10 appropriate substrate to a detectable product.

Alternatively, binding of a test compound to a galanin receptor-like polypeptide can be determined without labeling either of the interactants. For example, a micro-physiometer can be used to detect binding of a test compound with a galanin
15 receptor-like polypeptide. A microphysiometer (*e.g.*, Cytosensor™) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a test compound and a galanin receptor-like polypeptide (McConnell *et al.*, *Science* 257, 1906-1912, 1992).

20 Determining the ability of a test compound to bind to a galanin receptor-like polypeptide also can be accomplished using a technology such as real-time Bimolecular Interaction Analysis (BIA) (Sjolander & Urbaniczky, *Anal. Chem.* 63, 2338-2345, 1991, and Szabo *et al.*, *Curr. Opin. Struct. Biol.* 5, 699-705, 1995). BIA is a
25 technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore™). Changes in the optical phenomenon surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

30 In yet another aspect of the invention, a galanin receptor-like polypeptide can be used as a "bait protein" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent

5,283,317; Zervos *et al.*, *Cell* 72, 223-232, 1993; Madura *et al.*, *J. Biol. Chem.* 268, 12046-12054, 1993; Bartel *et al.*, *BioTechniques* 14, 920-924, 1993; Iwabuchi *et al.*, *Oncogene* 8, 1693-1696, 1993; and Brent W094/10300), to identify other proteins which bind to or interact with the galanin receptor-like polypeptide and modulate its activity.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. For example, in one construct, polynucleotide encoding a galanin receptor-like polypeptide can be fused to a polynucleotide encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct a DNA sequence that encodes an unidentified protein ("prey" or "sample") can be fused to a polynucleotide that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact *in vivo* to form a protein-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ), which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected, and cell colonies containing the functional transcription factor can be isolated and used to obtain the DNA sequence encoding the protein which interacts with the galanin receptor-like polypeptide.

It may be desirable to immobilize either the galanin receptor-like polypeptide (or polynucleotide) or the test compound to facilitate separation of bound from unbound forms of one or both of the interactants, as well as to accommodate automation of the assay. Thus, either the galanin receptor-like polypeptide (or polynucleotide) or the test compound can be bound to a solid support. Suitable solid supports include, but are not limited to, glass or plastic slides, tissue culture plates, microtiter wells, tubes, silicon chips, or particles such as beads (including, but not limited to, latex, polystyrene, or glass beads). Any method known in the art can be used to attach the galanin receptor-like polypeptide (or polynucleotide) or test compound to a solid

support, including use of covalent and non-covalent linkages, passive absorption, or pairs of binding moieties attached respectively to the polypeptide (or polynucleotide) or test compound and the solid support. Test compounds are preferably bound to the solid support in an array, so that the location of individual test compounds can be tracked. Binding of a test compound to a galanin receptor-like polypeptide (or polynucleotide) can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and microcentrifuge tubes.

In one embodiment, the galanin receptor-like polypeptide is a fusion protein comprising a domain that allows the galanin receptor-like polypeptide to be bound to a solid support. For example, glutathione-S-transferase fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, Mo.) or glutathione derivatized microtiter plates, which are then combined with the test compound or the test compound and the non-adsorbed galanin receptor-like polypeptide; the mixture is then incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components. Binding of the interactants can be determined either directly or indirectly, as described above. Alternatively, the complexes can be dissociated from the solid support before binding is determined.

Other techniques for immobilizing proteins or polynucleotides on a solid support also can be used in the screening assays of the invention. For example, either a galanin receptor-like polypeptide (or polynucleotide) or a test compound can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated galanin receptor-like polypeptides (or polynucleotides) or test compounds can be prepared from biotin-NHS(N-hydroxysuccinimide) using techniques well known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.) and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies which specifically bind to a galanin receptor-like polypeptide, polynucleotide, or a test

compound, but which do not interfere with a desired binding site, such as the active site of the galanin receptor-like polypeptide, can be derivatized to the wells of the plate. Unbound target or protein can be trapped in the wells by antibody conjugation.

5 Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies which specifically bind to the galanin receptor-like polypeptide or test compound, enzyme-linked assays which rely on detecting an activity of the galanin receptor-like polypeptide, and SDS gel electrophoresis under non-reducing
10 conditions.

Screening for test compounds which bind to a galanin receptor-like polypeptide or polynucleotide also can be carried out in an intact cell. Any cell which comprises a galanin receptor-like polypeptide or polynucleotide can be used in a cell-based assay
15 system. A galanin receptor-like polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Binding of the test compound to a galanin receptor-like polypeptide or polynucleotide is determined as described above.

20 Functional Assays

Test compounds can be tested for the ability to increase or decrease a galanin receptor-like GPCR activity of a galanin receptor-like polypeptide. Galanin receptor-like GPCR activity can be measured, for example, using methods described in the
25 specific examples, below. Galanin receptor-like GPCR activity can be measured after contacting either a purified galanin receptor-like polypeptide, a cell membrane preparation, or an intact cell with a test compound. A test compound which decreases galanin receptor-like GPCR activity by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential agent for
30 decreasing galanin receptor-like GPCR activity. A test compound which increases galanin receptor-like GPCR activity by at least about 10, preferably about 50, more

preferably about 75, 90, or 100% is identified as a potential agent for increasing galanin receptor-like GPCR activity.

5 One such screening procedure involves the use of melanophores which are transfected to express a galanin receptor-like polypeptide. Such a screening technique is described in PCT WO 92/01810 published Feb. 6, 1992. Thus, for example, such an assay may be employed for screening for a compound which inhibits activation of the receptor polypeptide of the present invention by contacting the melanophore cells which encode the receptor with both the receptor ligand and a compound to be
10 screened. Inhibition of the signal generated by the ligand indicates that a compound is a potential antagonist for the receptor, *i.e.*, inhibits activation of the receptor. The screen may be employed for identifying a compound which activates the receptor by contacting such cells with compounds to be screened and determining whether each compound generates a signal, *i.e.*, activates the receptor.

15 Other screening techniques include the use of cells which express galanin receptor-like polypeptide (for example, transfected CHO cells) in a system which measures extracellular pH changes caused by receptor activation (*see, e.g.*, Science 246, 181-296 (1989)). For example, compounds may be contacted with a cell which expresses
20 the receptor polypeptide of the present invention and a second messenger response, *e.g.*, signal transduction or pH changes, can be measured to determine whether the potential compound activates or inhibits the receptor.

25 Another such screening technique involves introducing RNA encoding a galanin receptor-like polypeptide into *Xenopus* oocytes to transiently express the receptor. The receptor oocytes can then be contacted with the receptor ligand and a compound to be screened, followed by detection of inhibition or activation of a calcium signal in the case of screening for compounds which are thought to inhibit activation of the receptor.

30

Another screening technique involves expressing a galanin receptor-like polypeptide in cells in which the receptor is linked to a phospholipase C or D. Such cells include endothelial cells, smooth muscle cells, embryonic kidney cells, etc. The screening may be accomplished as described above by quantifying the degree of activation of the receptor from changes in the phospholipase activity.

Gene Expression

In another embodiment, test compounds which increase or decrease galanin receptor-like GPCR gene expression are identified. A galanin receptor-like polynucleotide is contacted with a test compound, and the expression of an RNA or polypeptide product of the galanin receptor-like polynucleotide is determined. The level of expression of appropriate mRNA or polypeptide in the presence of the test compound is compared to the level of expression of mRNA or polypeptide in the absence of the test compound. The test compound can then be identified as a modulator of expression based on this comparison. For example, when expression of mRNA or polypeptide is greater in the presence of the test compound than in its absence, the test compound is identified as a stimulator or enhancer of the mRNA or polypeptide expression. Alternatively, when expression of the mRNA or polypeptide is less in the presence of the test compound than in its absence, the test compound is identified as an inhibitor of the mRNA or polypeptide expression.

The level of galanin receptor-like GPCR mRNA or polypeptide expression in the cells can be determined by methods well known in the art for detecting mRNA or polypeptide. Either qualitative or quantitative methods can be used. The presence of polypeptide products of a galanin receptor-like polynucleotide can be determined, for example, using a variety of techniques known in the art, including immunochemical methods such as radioimmunoassay, Western blotting, and immunohistochemistry. Alternatively, polypeptide synthesis can be determined *in vivo*, in a cell culture, or in an *in vitro* translation system by detecting incorporation of labeled amino acids into a galanin receptor-like polypeptide.

Such screening can be carried out either in a cell-free assay system or in an intact cell. Any cell which expresses a galanin receptor-like polynucleotide can be used in a cell-based assay system. The galanin receptor-like polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Either a primary culture or an established cell line can be used.

Pharmaceutical Compositions

The invention also provides pharmaceutical compositions which can be administered to a patient to achieve a therapeutic effect. Pharmaceutical compositions of the invention can comprise, for example, a galanin receptor-like polypeptide, galanin receptor-like polynucleotide, antibodies which specifically bind to a galanin receptor-like polypeptide, or mimetics, agonists, antagonists, or inhibitors of a galanin receptor-like polypeptide activity. The compositions can be administered alone or in combination with at least one other agent, such as stabilizing compound, which can be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions can be administered to a patient alone, or in combination with other agents, drugs or hormones.

In addition to the active ingredients, these pharmaceutical compositions can contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Pharmaceutical compositions of the invention can be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, parenteral, topical, sublingual, or rectal means. Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be

formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

5 Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, 10 hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents can be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate. •

15 Dragee cores can be used in conjunction with suitable coatings, such as concentrated sugar solutions, which also can contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments can be added to the tablets or dragee coatings for product identification or to characterize the quantity 20 of active compound, *i.e.*, dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a 25 filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds can be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

30 Pharmaceutical formulations suitable for parenteral administration can be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks'

solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions can contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds can be prepared as appropriate oily injection
5 suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Non-lipid polycationic amino polymers also can be used for delivery. Optionally, the suspension also can contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated
10 solutions. For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention can be manufactured in a
15 manner that is known in the art, *e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. The pharmaceutical composition can be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in
20 aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation can be a lyophilized powder which can contain any or all of the following: 1-50 mM histidine, 0.1%-2% sucrose, and 2-7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

25 Further details on techniques for formulation and administration can be found in the latest edition of REMINGTON'S PHARMACEUTICAL SCIENCES (Maack Publishing Co., Easton, Pa.). After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. Such labeling would include amount, frequency, and method of
30 administration.

Diagnostic and Therapeutic Indications and Methods

G-protein coupled receptors are ubiquitous in the mammalian host and are responsible for many biological functions, including many pathologies. Accordingly, it is desirable to find compounds and drugs which stimulate a G-protein coupled receptor on the one hand and which can inhibit the function of a G-protein coupled receptor on the other hand. For example, compounds which activate the G-protein coupled receptor may be employed for therapeutic purposes, such as the treatment of asthma, Parkinson's disease, acute heart failure, urinary retention, and osteoporosis. In particular, compounds which activate the receptors of the present invention are useful in treating various cardiovascular ailments such as caused by the lack of pulmonary blood flow or hypertension. In addition these compounds may also be used in treating various physiological disorders relating to abnormal control of fluid and electrolyte homeostasis and in diseases associated with abnormal angiotensin-induced aldosterone secretion.

In general, compounds which inhibit activation of the G-protein coupled receptor may be employed for a variety of therapeutic purposes, for example, for the treatment of hypotension and/or hypertension, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders including schizophrenia, manic excitement, depression, delirium, dementia or severe mental retardation, dyskinesias, such as Huntington's disease or Tourett's syndrome, among others. Compounds which inhibit G-protein coupled receptors have also been useful in reversing endogenous anorexia and in the control of bulimia.

Human galanin receptor-like GPCRs provide therapeutic targets for treating and diagnosing various diseases and abnormalities connected with these receptors. Galanin is co-released with norepinephrine from sympathetic nerve terminals, which suggests that galanin could act via galanin receptors in the periphery to modulate nearly every physiological process controlled by sympathetic innervation. Additional therapeutic indications include diabetes, hypertension, cancer, obesity,

osteoporosis, cardiovascular disorders, regulation of growth hormone release, regulation of fertility, gastric ulcers, gastrointestinal motility/transit/absorption/-secretion, glaucoma, inflammation, immune disorders, respiratory disorders (e.g. asthma, emphysema). See U.S. Patent 5,972,624.

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Galanin may also play a role in mediating effects on cognition, analgesia, neuroendocrine regulation, control of insulin release, and control of feeding behavior. Galanin receptor agonists may be useful as analgesic agents in the spinal cord. A galanin antagonist may be effective in ameliorating the symptoms of Alzheimer's disease.

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Human galanin receptor-like GPCRs provide a potential target for treating cancer. Cancer is a disease fundamentally caused by oncogenic cellular transformation. There are several hallmarks of transformed cells that distinguish them from their normal counterparts and underlie the pathophysiology of cancer. These include uncontrolled cellular proliferation, unresponsiveness to normal death-inducing signals (immortalization), increased cellular motility and invasiveness, increased ability to recruit blood supply through induction of new blood vessel formation (angiogenesis), genetic instability, and dysregulated gene expression. Various combinations of these aberrant physiologies, along with the acquisition of drug-resistance frequently lead to an intractable disease state in which organ failure and patient death ultimately ensue.

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Most standard cancer therapies target cellular proliferation and rely on the differential proliferative capacities between transformed and normal cells for their efficacy. This approach is hindered by the facts that several important normal cell types are also highly proliferative and that cancer cells frequently become resistant to these agents. Thus, the therapeutic indices for traditional anti-cancer therapies rarely exceed 2.0.

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The advent of genomics-driven molecular target identification has opened up the possibility of identifying new cancer-specific targets for therapeutic intervention that will provide safer, more effective treatments for cancer patients. Thus, newly discovered tumor-associated genes and their products can be tested for their role(s) in disease and used as tools to discover and develop innovative therapies. Genes playing important roles in any of the physiological processes outlined above can be characterized as cancer targets.

Genes or gene fragments identified through genomics can readily be expressed in one or more heterologous expression systems to produce functional recombinant proteins. These proteins are characterized *in vitro* for their biochemical properties and then used as tools in high-throughput molecular screening programs to identify chemical modulators of their biochemical activities. Agonists and/or antagonists of target protein activity can be identified in this manner and subsequently tested in cellular and *in vivo* disease models for anti-cancer activity. Optimization of lead compounds with iterative testing in biological models and detailed pharmacokinetic and toxicological analyses form the basis for drug development and subsequent testing in humans.

Human galanin receptor-like GPCR also provides a potential target for treating obesity and other eating disorders. Obesity and overweight are defined as an excess of body fat relative to lean body mass. An increase in caloric intake or a decrease in energy expenditure or both can bring about this imbalance leading to surplus energy being stored as fat. Obesity is associated with important medical morbidities and an increase in mortality. The causes of obesity are poorly understood and may be due to genetic factors, environmental factors or a combination of the two to cause a positive energy balance. In contrast, anorexia and cachexia are characterized by an imbalance in energy intake versus energy expenditure leading to a negative energy balance and weight loss. Agents that either increase energy expenditure and/or decrease energy intake, absorption or storage would be useful for treating obesity, overweight, and associated comorbidities. Agents that either increase energy intake and/or decrease

energy expenditure or increase the amount of lean tissue would be useful for treating cachexia, anorexia and wasting disorders.

5 This gene, translated proteins and agents which modulate this gene or portions of the gene or its products are useful for treating obesity, overweight, anorexia, cachexia, wasting disorders, appetite suppression, appetite enhancement, increases or decreases in satiety, modulation of body weight, and/or other eating disorders such as bulimia. Also this gene, translated proteins and agents which modulate this gene or portions of the gene or its products are useful for treating obesity/overweight-associated
10 comorbidities including hypertension; type 2 diabetes; coronary artery disease; hyperlipidemia; stroke; gallbladder disease; gout; osteoarthritis; sleep apnea and respiratory problems; some types of cancer including endometrial, breast, prostate and colon; thrombotic disease; polycystic ovarian syndrome; reduced fertility; complications of pregnancy; menstrual irregularities; hirsutism; stress incontinence
15 and depression.

Feeding behavior can be modified by administering to a mammal, preferably a human, an amount of a compound which is a galanin receptor-like GPCR agonist or antagonist effective to increase or decrease the consumption of food so as to thereby
20 modify feeding behavior. In one embodiment, the compound is a galanin receptor-like GPCR antagonist and the amount is effective to decrease the consumption of food by the subject. In another embodiment the compound is administered in combination with food. In yet another embodiment the compound is a galanin receptor-like GPCR agonist and the amount is effective to increase the consumption
25 of food by the subject. In a still further embodiment, the compound is administered in combination with food. Thus, an antagonist of the human galanin receptor-like GPCR can be administered to a mammal, including a human, to treat obesity or bulimia. An agonist of the human galanin receptor-like GPCR can be administered to treat anorexia.

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Diabetes also can be potentially treated by regulating the activity of human galanin receptor-like GPCR. Diabetes mellitus is a common metabolic disorder characterized by an abnormal elevation in blood glucose, alterations in lipids and abnormalities (complications) in the cardiovascular system, eye, kidney and nervous system. Diabetes is divided into two separate diseases: type 1 diabetes (juvenile onset) that results from a loss of cells which make and secrete insulin, and type 2 diabetes (adult onset) which is caused by a defect in insulin secretion and a defect in insulin action.

10 Type 1 diabetes is initiated by an autoimmune reaction that attacks the insulin secreting cells (beta cells) in the pancreatic islets. Agents that prevent this reaction from occurring or that stop the reaction before destruction of the beta cells has been accomplished are potential therapies for this disease. Other agents that induce beta cell proliferation and regeneration are also potential therapies.

15 Type II diabetes is the most common of the two diabetic conditions (6% of the population). The defect in insulin secretion is an important cause of the diabetic condition and results from an inability of the beta cell to properly detect and respond to rises in blood glucose levels with insulin release. Therapies that increase the response by the beta cell to glucose would offer an important new treatment for this disease.

20 The defect in insulin action in Type II diabetic subjects is another target for therapeutic intervention. Agents that increase the activity of the insulin receptor in muscle, liver and fat will cause a decrease in blood glucose and a normalization of plasma lipids. The receptor activity can be increased by agents that directly stimulate the receptor or that increase the intracellular signals from the receptor. Other therapies can directly activate the cellular end process, *i.e.* glucose transport or various enzyme systems, to generate an insulin-like effect and therefore a produce beneficial outcome. Because overweight subjects have a greater susceptibility to Type II diabetes, any agent that reduces body weight is a possible therapy.

Both Type I and Type diabetes can be treated with agents that mimic insulin action or that treat diabetic complications by reducing blood glucose levels. Likewise agents that reduces new blood vessel growth can be used to treat the eye complications that develop in both diseases.

Osteoporosis, too, can potentially be treated by regulating human galanin receptor-like GPCR. Osteoporosis is a disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk. It is the most common human metabolic bone disorder. Established osteoporosis includes the presence of fractures.

Bone turnover occurs by the action of two major effector cell types within bone: the osteoclast, which is responsible for bone resorption, and the osteoblast, which synthesizes and mineralizes bone matrix. The actions of osteoclasts and osteoblasts are highly coordinated. Osteoclast precursors are recruited to the site of turnover; they differentiate and fuse to form mature osteoclasts which then resorb bone. Attached to the bone surface, osteoclasts produce an acidic microenvironment in a tightly defined junction between the specialized osteoclast border membrane and the bone matrix, thus allowing the localized solubilization of bone matrix. This in turn facilitate the proteolysis of demineralized bone collagen. Matrix degradation is thought to release matrix-associated growth factor and cytokines, which recruit osteoblasts in a temporally and spatially controlled fashion. Osteoblasts synthesize and secrete new bone matrix proteins, and subsequently mineralize this new matrix. In the normal skeleton this is a physiological process which does not result in a net change in bone mass. In pathological states, such as osteoporosis, the balance between resorption and formation is altered such that bone loss occurs.

The osteoclast itself is the direct or indirect target of all currently available osteoporosis agents with the possible exception of fluoride. Antiresorptive therapy prevents further bone loss in treated individuals. Osteoblasts are derived from

5 multipotent stem cells which reside in bone marrow and also gives rise to adipocytes, chondrocytes, fibroblasts and muscle cells. Selective enhancement of osteoblast activity is a highly desirable goal for osteoporosis therapy since it would result in an increase in bone mass, rather than a prevention of further bone loss. An effective anabolic therapy would be expected to lead to a significantly greater reduction in fracture risk than currently available treatments.

10 The agonists or antagonists to the newly discovered polypeptides may act as antiresorptive by directly altering the osteoclast differentiation, osteoclast adhesion to the bone matrix or osteoclast function of degrading the bone matrix. The agonists or antagonists could indirectly alter the osteoclast function by interfering in the synthesis and/or modification of effector molecules of osteoclast differentiation or function such as cytokines, peptide or steroid hormones, proteases, etc.

15 The agonists or antagonists to the newly discovered polypeptides may act as anabolics by directly enhancing the osteoblast differentiation and /or its bone matrix forming function. The agonists or antagonists could also indirectly alter the osteoblast function by enhancing the synthesis of growth factors, peptide or steroid hormones or decreasing the synthesis of inhibitory molecules.

20 The agonists and antagonists may be used to mimic, augment or inhibit the action of the newly discovered polypeptides which may be useful to treat osteoporosis, Paget's disease, degradation of bone implants particularly dental implants.

25 This invention further pertains to the use of novel agents identified by the screening assays described above. Accordingly, it is within the scope of this invention to use a test compound identified as described herein in an appropriate animal model. For example, an agent identified as described herein (e.g., a modulating agent, an antisense nucleic acid molecule, a specific antibody, ribozyme, or a protein-binding partner) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as

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described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

5 A reagent which affects galanin receptor-like GPCR activity can be administered to a human cell, either *in vitro* or *in vivo*, to reduce galanin receptor-like GPCR activity. The reagent preferably binds to an expression product of a human galanin receptor-like GPCR gene. If the expression product is a protein, the reagent is preferably an antibody. For treatment of human cells *ex vivo*, an antibody can be added to a
10 preparation of stem cells which have been removed from the body. The cells can then be replaced in the same or another human body, with or without clonal propagation, as is known in the art.

In one embodiment, the reagent is delivered using a liposome. Preferably, the
15 liposome is stable in the animal into which it has been administered for at least about 30 minutes, more preferably for at least about 1 hour, and even more preferably for at least about 24 hours. A liposome comprises a lipid composition that is capable of targeting a reagent, particularly a polynucleotide, to a particular site in an animal, such as a human. Preferably, the lipid composition of the liposome is capable of
20 targeting to a specific organ of an animal, such as the lung, liver, spleen, heart brain, lymph nodes, and skin.

A liposome useful in the present invention comprises a lipid composition that is capable of fusing with the plasma membrane of the targeted cell to deliver its
25 contents to the cell. Preferably, the transfection efficiency of a liposome is about 0.5 μg of DNA per 16 nmole of liposome delivered to about 10^6 cells, more preferably about 1.0 μg of DNA per 16 nmol of liposome delivered to about 10^6 cells, and even more preferably about 2.0 μg of DNA per 16 nmol of liposome delivered to about 10^6 cells. Preferably, a liposome is between about 100 and 500 nm, more

preferably between about 150 and 450 nm, and even more preferably between about 200 and 400 nm in diameter.

5 Suitable liposomes for use in the present invention include those liposomes standardly used in, for example, gene delivery methods known to those of skill in the art. More preferred liposomes include liposomes having a polycationic lipid composition and/or liposomes having a cholesterol backbone conjugated to polyethylene glycol. Optionally, a liposome comprises a compound capable of targeting the liposome to a tumor cell, such as a tumor cell ligand exposed on the
10 outer surface of the liposome.

Complexing a liposome with a reagent such as an antisense oligonucleotide or ribozyme can be achieved using methods which are standard in the art (see, for example, U.S. Patent 5,705,151). Preferably, from about 0.1 μ g to about 10 μ g of
15 polynucleotide is combined with about 8 nmol of liposomes, more preferably from about 0.5 μ g to about 5 μ g of polynucleotides are combined with about 8 nmol liposomes, and even more preferably about 1.0 μ g of polynucleotides is combined with about 8 nmol liposomes.

20 In another embodiment, antibodies can be delivered to specific tissues *in vivo* using receptor-mediated targeted delivery. Receptor-mediated DNA delivery techniques are taught in, for example, Findeis *et al.* *Trends in Biotechnol.* 11, 202-05 (1993); Chiou *et al.*, GENE THERAPEUTICS: METHODS AND APPLICATIONS OF DIRECT GENE TRANSFER (J.A. Wolff, ed.) (1994); Wu & Wu, *J. Biol. Chem.* 263, 621-24 (1988);
25 Wu *et al.*, *J. Biol. Chem.* 269, 542-46 (1994); Zenke *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 87, 3655-59 (1990); Wu *et al.*, *J. Biol. Chem.* 266, 338-42 (1991).

Determination of a Therapeutically Effective Dose

The determination of a therapeutically effective dose is well within the capability of those skilled in the art. A therapeutically effective dose refers to that amount of active ingredient which increases or decreases galanin receptor-like GPCR activity relative to the galanin receptor-like GPCR activity which occurs in the absence of the therapeutically effective dose.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually mice, rabbits, dogs, or pigs. The animal model also can be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

Therapeutic efficacy and toxicity, *e.g.*, ED_{50} (the dose therapeutically effective in 50% of the population) and LD_{50} (the dose lethal to 50% of the population), can be determined by standard pharmaceutical procedures in cell cultures or experimental animals. The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD_{50}/ED_{50} .

Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active ingredient or to maintain the desired effect. Factors which can be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and

tolerance/response to therapy. Long-acting pharmaceutical compositions can be administered every 3 to 4 days, every week, or once every two weeks depending on the half-life and clearance rate of the particular formulation.

5 Normal dosage amounts can vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of
10 polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

If the reagent is a single-chain antibody, polynucleotides encoding the antibody can be constructed and introduced into a cell either *ex vivo* or *in vivo* using well-
15 established techniques including, but not limited to, transferrin-polycation-mediated DNA transfer, transfection with naked or encapsulated nucleic acids, liposome-mediated cellular fusion, intracellular transportation of DNA-coated latex beads, protoplast fusion, viral infection, electroporation, "gene gun," and DEAE- or calcium phosphate-mediated transfection.

20 Effective *in vivo* dosages of an antibody are in the range of about 5 μ g to about 50 μ g/kg, about 50 μ g to about 5 mg/kg, about 100 μ g to about 500 μ g/kg of patient body weight, and about 200 to about 250 μ g/kg of patient body weight. For administration of polynucleotides encoding single-chain antibodies, effective *in vivo*
25 dosages are in the range of about 100 ng to about 200 ng, 500 ng to about 50 mg, about 1 μ g to about 2 mg, about 5 μ g to about 500 μ g, and about 20 μ g to about 100 μ g of DNA.

If the expression product is mRNA, the reagent is preferably an antisense oligo-
30 nucleotide or a ribozyme. Polynucleotides which express antisense oligonucleotides

or ribozymes can be introduced into cells by a variety of methods, as described above.

5 Preferably, a reagent reduces expression of a galanin receptor-like GPCR gene or the activity of a galanin receptor-like polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the reagent. The effectiveness of the mechanism chosen to decrease the level of expression of a galanin receptor-like GPCR gene or the activity of a galanin receptor-like polypeptide can be assessed using methods well known in the art, such as hybridization
10 of nucleotide probes to galanin receptor-like GPCR-specific mRNA, quantitative RT-PCR, immunologic detection of a galanin receptor-like polypeptide, or measurement of galanin receptor-like GPCR activity.

15 In any of the embodiments described above, any of the pharmaceutical compositions of the invention can be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy can be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents can act synergistically to effect the treatment or prevention of the various disorders described above.
20 Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

Any of the therapeutic methods described above can be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows,
25 horses, rabbits, monkeys, and most preferably, humans.

Diagnostic Methods

30 The invention also relates to the use of galanin receptor-like GPCR genes as part of a diagnostic assay for detecting diseases and abnormalities or susceptibility to diseases and abnormalities related to the presence of mutations in the nucleic acid sequences

which encode a galanin receptor-like polypeptide. Such diseases, by way of example, are related to cell transformation, such as tumors and cancers, and various cardiovascular disorders, including hypertension and hypotension, as well as diseases arising from abnormal blood flow, abnormal angiotensin-induced aldosterone secretion, and other abnormal control of fluid and electrolyte homeostasis.

Using polynucleotides disclosed herein, differences can be determined between the cDNA or genomic sequence of individuals afflicted with a disease and normal individuals. If a mutation is observed in some or all of the afflicted individuals but not in normal individuals, then the mutation is likely to be the causative agent of the disease.

Sequence differences between a reference gene and a gene having mutations can be revealed by the direct DNA sequencing method. In addition, cloned DNA segments can be employed as probes to detect specific DNA segments. The sensitivity of this method is greatly enhanced when combined with PCR. For example, a sequencing primer can be used with a double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures using radiolabeled nucleotides or by automatic sequencing procedures using fluorescent tags.

Genetic testing based on DNA sequence differences can be carried out by detection of alteration in electrophoretic mobility of DNA fragments in gels with or without denaturing agents. Small sequence deletions and insertions can be visualized by high resolution gel electrophoresis. DNA fragments of different sequences can be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (*see, e.g., Myers et al., Science* 230, 1242, 1985). Sequence changes at specific locations can also be revealed by nuclease protection assays, such as RNase and S 1 protection or the chemical cleavage method (*e.g., Cotton et al., Proc. Natl. Acad. Sci. USA* 85, 4397-4401,

1985). Thus, the detection of a specific DNA sequence can be performed by methods such as hybridization, RNase protection, chemical cleavage, direct DNA sequencing or the use of restriction enzymes and Southern blotting of genomic DNA. In addition to direct methods such as gel-electrophoresis and DNA sequencing, mutations can also be detected by in situ analysis.

Another embodiment is a diagnostic assay for detecting altered levels of galanin receptor-like polypeptides in various tissues. Assays used to detect levels of the receptor polypeptides in a sample derived from a host are well known to those of skill in the art and include radioimmunoassays, competitive binding assays, Western blot analysis, and ELISA assays.

All patents and patent applications cited in this disclosure are expressly incorporated herein by reference. The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples which are provided for purposes of illustration only and are not intended to limit the scope of the invention.

EXAMPLE 1

Detection of galanin receptor-like GPCR activity

The polynucleotide of SEQ ID NO. 1 is inserted into the expression vector pCEV4 and the expression vector pCEV4-galanin receptor-like GPCR polypeptide obtained is transfected into human embryonic kidney 293 cells. The cells are scraped from a culture flask into 5 ml of Tris HCl, 5 mM EDTA, pH 7.5, and lysed by sonication. Cell lysates are centrifuged at 1000 rpm for 5 minutes at 4°C. The supernatant is centrifuged at 30,000 x g for 20 minutes at 4 °C. The pellet is suspended in binding buffer containing 50 mM Tris HCl, 5 mM MgSO₄, 1 mM EDTA, 100 mM NaCl, pH 7.5, supplemented with 0.1 % BSA, 2 µg/ml aprotinin, 0.5 mg/ml leupeptin, and

10 µg/ml phosphoramidon. Optimal membrane suspension dilutions, defined as the protein concentration required to bind less than 10 % of an added radioligand, i.e. ¹²⁵I-labeled galanin, are added to 96-well polypropylene microtiter plates containing ligand, non-labeled peptides, and binding buffer to a final volume of 250 µl.

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In equilibrium saturation binding assays, membrane preparations are incubated in the presence of increasing concentrations (0.1 nM to 4 nM) of ¹²⁵I ligand.

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Binding reaction mixtures are incubated for one hour at 30°C. The reaction is stopped by filtration through GF/B filters treated with 0.5% polyethyleneimine, using a cell harvester. Radioactivity is measured by scintillation counting, and data are analyzed by a computerized non-linear regression program. Non-specific binding is defined as the amount of radioactivity remaining after incubation of membrane protein in the presence of 100 nM of unlabeled peptide. Protein concentration is measured by the Bradford method using Bio-Rad Reagent, with bovine serum albumin as a standard. The galanin receptor-like GPCR activity of the polypeptide comprising the amino acid sequence of SEQ ID NO. 2 is demonstrated

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EXAMPLE 2

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Radioligand binding assays

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Transfected cells from culture flasks are scraped into 5 ml of Tris-HCl, 5 mM EDTA, pH 7.5, and lysed by sonication. The cell lysates are centrifuged at 1000 rpm for 5 min. at 4°C, and the supernatant is centrifuged at 30,000 x g for 20 min. at 4°C. The pellet is suspended in binding buffer (50 mM Tris-HCl, 5 mM MgSO₄, 1 mM EDTA, 100 mM NaCl at pH 7.5 supplemented with 0.1% BSA, 2 µg/ml aprotinin, 0.5 mg/ml leupeptin, and 10 µg/ml phosphoramidon). Optimal membrane suspension dilutions, defined as the protein concentration required to bind less than 10% of the added radioligand, i.e. galanin, are added to 96-well polypropylene microtiter plates

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containing ^{125}I -labeled peptide, non-labeled peptides, and binding buffer to a final volume of 250 μl .

In equilibrium saturation binding assays membrane preparations are incubated in the presence of increasing concentrations (0.1 nM to 4 nM) of ^{125}I -porcine galanin (specific activity 2200 Ci/mmol). The binding affinities of the different galanin analogs are determined in equilibrium competition binding assays, using 0.1 nM ^{125}I -porcine galanin in the presence of twelve different concentrations of the displacing ligands. Binding reaction mixtures are incubated for 1 hr at 30°C, and the reaction is stopped by filtration through GF/B filters treated with 0.5% polyethyleneimine, using a cell harvester. Radioactivity is measured by scintillation counting and data are analyzed by a computerized non-linear regression program. Non-specific binding is defined as the amount of radioactivity remaining after incubation of membrane protein in the presence of 100 nM of unlabeled porcine galanin. Protein concentration is measured by the Bradford method using Bio-Rad Reagent, with bovine serum albumin as a standard.

EXAMPLE 3

Effect of a test compound on galanin receptor-like GPCR-mediated cyclic AMP formation

The receptor-mediated inhibition of cyclic AMP (cAMP) formation is assayed in LM(tk-) cells expressing rat GALR1 and human galanin receptor-like GPCR receptors. Cells are plated in 96 well plates and incubated in Dulbecco's phosphate buffered saline (PBS) supplemented with 10 mM HEPES, 5 mM theophylline, 2 $\mu\text{g/ml}$ aprotinin, 0.5 mg/ml leupeptin, and 10 $\mu\text{g/ml}$ phosphoramidon for 20 min at 37°C, in 5% CO_2 . Galanin or test compounds are added and incubated for an additional 10 min at 37°C. The medium is aspirated, and the reaction is stopped by the addition of 100 mM HCl. The plates are stored at 4°C for 15 min, and the cAMP

content in the stopping solution is measured by radioimmunoassay. Radioactivity is quantified using a gamma counter equipped with data reduction software.

EXAMPLE 4

Effect of a test compound on the mobilization of intracellular calcium

Intracellular free calcium concentration can be measured by microspectrofluorometry using the fluorescent indicator dye Fura-2/AM (Bush *et al.*, *J. Neurochem.* 57, 562-74, 1991). Stably transfected cells are seeded onto a 35 mm culture dish containing a glass coverslip insert. Cells are washed with HBS, incubated with a test compound, and loaded with 100 μ l of Fura-2/AM (10 μ M) for 20-40 minutes. After washing with HBS to remove the Fura-2/AM solution, cells are equilibrated in HBS for 10-20 minutes. Cells are then visualized under the 40X objective of a Leitz Fluovert FS microscope.

Fluorescence emission is determined at 510 nm, with excitation wavelengths alternating between 340 nm and 380 nm. Raw fluorescence data are converted to calcium concentrations using standard calcium concentration curves and software analysis techniques. A test compound which increases the fluorescence by at least 15% relative to fluorescence in the absence of a test compound is identified as a compound which mobilizes intracellular calcium.

EXAMPLE 5

Effect of a test compound on phosphoinositide metabolism

LM(tk-) cells which stably express human galanin receptor-like GPCR cDNA are plated in 96-well plates and grown to confluence. The day before the assay, the growth medium is changed to 100 μ l of medium containing 1% serum and 0.5 μ Ci

³H-myoinositol. The plates are incubated overnight in a CO₂ incubator (5% CO₂ at 37°C). Immediately before the assay, the medium is removed and replaced by 200 µl of PBS containing 10 mM LiCl, and the cells are equilibrated with the new medium for 20 minutes. During this interval, cells also are equilibrated with antagonist, added as a 10 µl aliquot of a 20-fold concentrated solution in PBS.

The ³H-inositol phosphate accumulation from inositol phospholipid metabolism is started by adding 10 µl of a solution containing a test compound. To the first well 10 µl are added to measure basal accumulation. Eleven different concentrations of test compound are assayed in the following 11 wells of each plate row. All assays are performed in duplicate by repeating the same additions in two consecutive plate rows.

The plates are incubated in a CO₂ incubator for one hour. The reaction is terminated by adding 15 µl of 50% v/v trichloroacetic acid (TCA), followed by a 40 minute incubation at 4°C. After neutralizing TCA with 40 µl of 1 M Tris, the content of the wells is transferred to a Multiscreen HV filter plate (Millipore) containing Dowex AG1-X8 (200-400 mesh, formate form). The filter plates are prepared by adding 200 µl of Dowex AG1-X8 suspension (50% v/v, water:resin) to each well. The filter plates are placed on a vacuum manifold to wash or elute the resin bed. Each well is washed 2 times with 200 µl of water, followed by 2 x 200 µl of 5 mM sodium tetraborate/60 mM ammonium formate.

The ³H-IPs are eluted into empty 96-well plates with 200 µl of 1.2 M ammonium formate/0.1 formic acid. The content of the wells is added to 3 ml of scintillation cocktail, and radioactivity is determined by liquid scintillation counting.

EXAMPLE 6

30 *Receptor Binding Methods*

Standard Binding Assays. Binding assays are carried out in a binding buffer containing 50 mM HEPES, pH 7.4, 0.5% BSA, and 5 mM $MgCl_2$. The standard assay for radioligand binding to membrane fragments comprising GPCR homolog 2 polypeptides is carried out as follows in 96 well microtiter plates (*e.g.*, Dynatech Immulon II Removawell plates). Radioligand is diluted in binding buffer+ PMSF/Baci to the desired cpm per 50 μ l, then 50 μ l aliquots are added to the wells. For non-specific binding samples, 5 μ l of 40 μ M cold ligand also is added per well. Binding is initiated by adding 150 μ l per well of membrane diluted to the desired concentration (10-30 μ g membrane protein/well) in binding buffer+ PMSF/Baci. Plates are then covered with Linbro mylar plate sealers (Flow Labs) and placed on a Dynatech Microshaker II. Binding is allowed to proceed at room temperature for 1-2 hours and is stopped by centrifuging the plate for 15 minutes at 2,000 x g. The supernatants are decanted, and the membrane pellets are washed once by addition of 200 μ l of ice cold binding buffer, brief shaking, and recentrifugation. The individual wells are placed in 12 x 75 mm tubes and counted in an LKB Gammamaster counter (78% efficiency). Specific binding by this method is identical to that measured when free ligand is removed by rapid (3-5 seconds) filtration and washing on polyethyleneimine-coated glass fiber filters.

Three variations of the standard binding assay are also used.

1. Competitive radioligand binding assays with a concentration range of cold ligand vs. 125 I-labeled ligand are carried out as described above with one modification. All dilutions of ligands being assayed are made in 40X PMSF/Baci to a concentration 40X the final concentration in the assay. Samples of peptide (5 μ l each) are then added per microtiter well. Membranes and radioligand are diluted in binding buffer without protease inhibitors. Radioligand is added and mixed with cold ligand, and then binding is initiated by addition of membranes.

2. Chemical cross-linking of radioligand with receptor is done after a binding step identical to the standard assay. However, the wash step is done with binding buffer minus BSA to reduce the possibility of non-specific cross-linking of radioligand with BSA. The cross-linking step is carried out as described below.
3. Larger scale binding assays to obtain membrane pellets for studies on solubilization of receptor:ligand complex and for receptor purification are also carried out. These are identical to the standard assays except that (a) binding is carried out in polypropylene tubes in volumes from 1-250 ml, (b) concentration of membrane protein is always 0.5 mg/ml, and (c) for receptor purification, BSA concentration in the binding buffer is reduced to 0.25%, and the wash step is done with binding buffer without BSA, which reduces BSA contamination of the purified receptor.

EXAMPLE 7

Chemical Cross-Linking of Radioligand to Receptor

After a radioligand binding step as described above, membrane pellets are re-suspended in 200 μ l per microtiter plate well of ice-cold binding buffer without BSA. Then 5 μ l per well of 4 mM N-5-azido-2-nitrobenzoyloxysuccinimide (ANB-NOS, Pierce) in DMSO is added and mixed. The samples are held on ice and UV-irradiated for 10 minutes with a Mineralight R-52G lamp (UVP Inc., San Gabriel, Calif.) at a distance of 5-10 cm. Then the samples are transferred to Eppendorf microfuge tubes, the membranes pelleted by centrifugation, supernatants removed, and membranes solubilized in Laemmli SDS sample buffer for polyacrylamide gel electrophoresis (PAGE). PAGE is carried out as described below. Radiolabeled

proteins are visualized by autoradiography of the dried gels with Kodak XAR film and DuPont image intensifier screens.

EXAMPLE 8

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Membrane Solubilization

Membrane solubilization is carried out in buffer containing 25 mM Tris , pH 8, 10% glycerol (w/v) and 0.2 mM CaCl₂ (solubilization buffer). The highly soluble detergents including Triton X-100, deoxycholate, deoxycholate:lysolecithin, CHAPS, and zwittergent are made up in solubilization buffer at 10% concentrations and stored as frozen aliquots. Lysolecithin is made up fresh because of insolubility upon freeze-thawing and digitonin is made fresh at lower concentrations due to its more limited solubility.

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To solubilize membranes, washed pellets after the binding step are resuspended free of visible particles by pipetting and vortexing in solubilization buffer at 100,000 x g for 30 minutes. The supernatants are removed and held on ice and the pellets are discarded.

20

EXAMPLE 9

Assay of Solubilized Receptors

After binding of ¹²⁵I ligands and solubilization of the membranes with detergent, the intact R:L complex can be assayed by four different methods. All are carried out on ice or in a cold room at 4-10°C.).

1. Column chromatography (Knuhtsen *et al.*, *Biochem. J.* 254, 641-647, 1988).
Sephadex G-50 columns (8 x 250 mm) are equilibrated with solubilization buffer containing detergent at the concentration used to solubilize membranes

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and 1 mg/ml bovine serum albumin. Samples of solubilized membranes (0.2-0.5 ml) are applied to the columns and eluted at a flow rate of about 0.7 ml/minute. Samples (0.18 ml) are collected. Radioactivity is determined in a gamma counter. Void volumes of the columns are determined by the elution volume of blue dextran. Radioactivity eluting in the void volume is considered bound to protein. Radioactivity eluting later, at the same volume as free ^{125}I ligands, is considered non-bound.

2. Polyethyleneglycol precipitation (Cuatrecasas, *Proc. Natl. Acad. Sci. USA* 69, 318-322, 1972). For a 100 μl sample of solubilized membranes in a 12 x 75 mm polypropylene tube, 0.5 ml of 1% (w/v) bovine gamma globulin (Sigma) in 0.1 M sodium phosphate buffer is added, followed by 0.5 ml of 25% (w/v) polyethyleneglycol (Sigma) and mixing. The mixture is held on ice for 15 minutes. Then 3 ml of 0.1 M sodium phosphate, pH 7.4, is added per sample. The samples are rapidly (1-3 seconds) filtered over Whatman GF/B glass fiber filters and washed with 4 ml of the phosphate buffer. PEG-precipitated receptor : ^{125}I -ligand complex is determined by gamma counting of the filters.
3. GFB/PEI filter binding (Bruns *et al.*, *Analytical Biochem.* 132, 74-81, 1983). Whatman GF/B glass fiber filters are soaked in 0.3% polyethyleneimine (PEI, Sigma) for 3 hours. Samples of solubilized membranes (25-100 μl) are replaced in 12 x 75 mm polypropylene tubes. Then 4 ml of solubilization buffer without detergent is added per sample and the samples are immediately filtered through the GFB/PEI filters (1-3 seconds) and washed with 4 ml of solubilization buffer. CPM of receptor : ^{125}I -ligand complex adsorbed to filters are determined by gamma counting.
4. Charcoal/Dextran (Paul and Said, *Peptides* 7[Suppl. 1], 147-149, 1986). Dextran T70 (0.5 g, Pharmacia) is dissolved in 1 liter of water, then 5 g of activated charcoal (Norit A, alkaline; Fisher Scientific) is added. The

suspension is stirred for 10 minutes at room temperature and then stored at 4°C. until use. To measure R:L complex, 4 parts by volume of charcoal/dextran suspension are added to 1 part by volume of solubilized membrane. The samples are mixed and held on ice for 2 minutes and then centrifuged for 2 minutes at 11,000 x g in a Beckman microfuge. Free radioligand is adsorbed charcoal/dextran and is discarded with the pellet. Receptor : ¹²⁵I-ligand complexes remain in the supernatant and are determined by gamma counting.

10 EXAMPLE 10

Receptor Purification

Binding of biotinyl-receptor to GH₄ C1 membranes is carried out as described above. Incubations are for 1 hour at room temperature. In the standard purification protocol, the binding incubations contain 10 nM Bio-S29. ¹²⁵I ligand is added as a tracer at levels of 5,000-100,000 cpm per mg of membrane protein. Control incubations contain 10 μM cold ligand to saturate the receptor with non-biotinylated ligand.

20 Solubilization of receptor:ligand complex also is carried out as described above, with 0.15% deoxycholate:lysolecithin in solubilization buffer containing 0.2 mM MgCl₂, to obtain 100,000 x g supernatants containing solubilized R:L complex.

25 Immobilized streptavidin (streptavidin cross-linked to 6% beaded agarose, Pierce Chemical Co.; "SA-agarose") is washed in solubilization buffer and added to the solubilized membranes as 1/30 of the final volume. This mixture is incubated with constant stirring by end-over-end rotation for 4-5 hours at 4-10 °C. Then the mixture is applied to a column and the non-bound material is washed through. Binding of radioligand to SA-agarose is determined by comparing cpm in the 100,000 x g supernatant with that in the column effluent after adsorption to SA-agarose. Finally,

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the column is washed with 12-15 column volumes of solubilization buffer+0.15% deoxycholate:lysolecithin +1/500 (vol/vol) 100 x 4pase.

5 The streptavidin column is eluted with solubilization buffer+0.1 mM EDTA+0.1 mM EGTA+0.1 mM GTP-gamma-S (Sigma)+0.15% (wt/vol) deoxycholate:lysolecithin +1/1000 (vol/vol) 100.times.4pase. First, one column volume of elution buffer is passed through the column and flow is stopped for 20-30 minutes. Then 3-4 more column volumes of elution buffer are passed through. All the eluates are pooled.

10 Eluates from the streptavidin column are incubated overnight (12-15 hours) with immobilized wheat germ agglutinin (WGA agarose, Vector Labs) to adsorb the receptor via interaction of covalently bound carbohydrate with the WGA lectin. The ratio (vol/vol) of WGA-agarose to streptavidin column eluate is generally 1:400. A range from 1:1000 to 1:200 also can be used. After the binding step, the resin is
15 pelleted by centrifugation, the supernatant is removed and saved, and the resin is washed 3 times (about 2 minutes each) in buffer containing 50 mM HEPES, pH 8, 5 mM MgCl₂ and 0.15% deoxycholate:lysolecithin. To elute the WGA-bound receptor, the resin is extracted three times by repeated mixing (vortex mixer on low speed) over a 15-30 minute period on ice, with 3 resin columns each time, of 10 mM
20 N-N'-N"-triacetylchitotriose in the same HEPES buffer used to wash the resin. After each elution step, the resin is centrifuged down and the supernatant is carefully removed, free of WGA-agarose pellets. The three, pooled eluates contain the final, purified receptor. The material non-bound to WGA contain G protein subunits specifically eluted from the streptavidin column, as well as non-specific
25 contaminants. All these fractions are stored frozen at -90°C.

EXAMPLE 11

Effect of a test compound on galanin receptor-like GPCR-mediated food intake

To determine whether a compound is a human galanin receptor-like GPCR antagonist, food intake in rats may be stimulated by administration of the human galanin receptor-like GPCR-selective peptide agonist [D-Trp₂]-galanin₁₋₂₉ through an intracerebroventricular (i.c.v.) cannula. A preferred anatomic location for injection is the hypothalamus, in particular, the paraventricular nucleus. Methods of cannulation and food intake measurements are well-known in the art, as are i.c.v. modes of administration (Kyrkouli *et al.*, 1990, Ogren *et al.*, 1992). To determine whether a test compound reduces [D-Trp₂]-galanin₁₋₂₉ stimulated food intake, the test compound is administered either simultaneously with the peptide, or separately, either through cannula, or by subcutaneous, intramuscular, or intraperitoneal injection, or more preferably, orally.

EXAMPLE 12

15 *Pharmacological characterization of galanin receptor-like GPCR*

The pharmacology of human galanin receptor-like GPCR is studied in COS-7 cells transiently transfected with a human galanin receptor-like GPCR-encoding cDNA. Membrane preparations of COS-7 cells transfected with K985 display specific binding to [¹²⁵I]porcine galanin. Scatchard analysis of equilibrium saturation binding data is used to determine K_d and B_{max}. The pharmacological properties of human galanin receptor-like GPCR cDNA are probed by measuring the binding affinities of a series of galanin analogs, and compared to those of the rat GALR1 receptor expressed in the same host cell line.

EXAMPLE 13

25 *Identification of a test compound which binds to a galanin receptor-like polypeptide*

30 Purified galanin receptor-like polypeptides comprising a glutathione-S-transferase protein and absorbed onto glutathione-derivatized wells of 96-well microtiter plates

are contacted with test compounds from a small molecule library at pH 7.0 in a physiological buffer solution. Galanin receptor-like polypeptides comprise an amino acid sequence shown in SEQ ID NO.2, 4 or 5. The test compounds comprise a fluorescent tag. The samples are incubated for 5 minutes to one hour. Control samples are incubated in the absence of a test compound.

The buffer solution containing the test compounds is washed from the wells. Binding of a test compound to a galanin receptor-like polypeptide is detected by fluorescence measurements of the contents of the wells. A test compound which increases the fluorescence in a well by at least 15% relative to fluorescence of a well in which a test compound was not incubated is identified as a compound which binds to a galanin receptor-like polypeptide.

EXAMPLE 14

Identification of a test compound which decreases human galanin receptor-like GPCR gene expression

A test compound is administered to a culture of human CHO cells which express human galanin receptor-like GPCR and incubated at 37°C for 10 to 45 minutes. A culture of the same type of cells incubated for the same time without the test compound provides a negative control.

RNA is isolated from the two cultures as described in Chirgwin *et al.*, *Biochem.* 18, 5294-99, 1979). Northern blots are prepared using 20 to 30 µg total RNA and hybridized with a ³²P-labeled galanin receptor-like GPCR-specific probe at 65°C in Express-hyb (CLONTECH). The probe comprises at least 11 contiguous nucleotides selected from SEQ ID NO.1 or 3. A test compound which decreases the galanin receptor-like GPCR-specific signal relative to the signal obtained in the absence of

the test compound is identified as an inhibitor of galanin receptor-like GPCR gene expression.

EXAMPLE 15

Treatment of asthma with a reagent which specifically binds to a human galanin receptor-like GPCR gene product

Synthesis of antisense galanin receptor-like GPCR oligonucleotides comprising at least 11 contiguous nucleotides selected from SEQ ID NO.1 or 3 is performed on a Pharmacia Gene Assembler series synthesizer using the phosphoramidite procedure (Uhlmann *et al.*, *Chem. Rev.* 90, 534-83, 1990). Following assembly and deprotection, oligonucleotides are ethanol-precipitated twice, dried, and suspended in phosphate-buffered saline (PBS) at the desired concentration. Purity of these oligonucleotides is tested by capillary gel electrophoreses and ion exchange HPLC. Endotoxin levels in the oligonucleotide preparation are determined using the *Limulus* Amebocyte Assay (Bang, *Biol. Bull. (Woods Hole, Mass.)* 105, 361-362, 1953).

The antisense oligonucleotides are administered to a patient with asthma. The severity of the patient's depression is decreased.

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CLAIMS

1. An isolated polynucleotide encoding a galanin receptor-like GPCR polypeptide and being selected from the group consisting of:

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- a) a polynucleotide encoding a galanin receptor-like GPCR polypeptide comprising an amino acid sequence selected from the group consisting of:

10

amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO. 2;

the amino acid sequence shown in SEQ ID NO. 2;

amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO. 4

the amino acid sequence shown in SEQ ID NO. 4;

15

amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO. 5; and

the amino acid sequence shown in SEQ ID NO.5.

20

- b) a polynucleotide comprising the sequence of SEQ ID NO. 1 or 3;

- c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and (b);

25

- d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code; and

30

- e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d).

2. An expression vector containing any polynucleotide of claim 1.

3. A host cell containing the expression vector of claim 2.
4. A substantially purified galanin receptor-like GPCR polypeptide encoded by
5 a polynucleotide of claim 1.
5. A method for producing a galanin receptor-like GPCR polypeptide, wherein
the method comprises the following steps:
 - 10 a) culturing the host cell of claim 3 under conditions suitable for the
expression of the galanin receptor-like GPCR polypeptide; and
 - b) recovering the galanin receptor-like GPCR polypeptide from the host,
15 cell culture.
6. A method for detection of a polynucleotide encoding a galanin receptor-like
GPCR polypeptide in a biological sample comprising the following steps:
 - 20 a) hybridizing any polynucleotide of claim 1 to a nucleic acid material of
a biological sample, thereby forming a hybridization complex; and
 - b) detecting said hybridization complex.
7. The method of claim 6, wherein before hybridization, the nucleic acid
25 material of the biological sample is amplified.
8. A method for the detection of a polynucleotide of claim 1 or a galanin
receptor-like GPCR polypeptide of claim 4 comprising the steps of:
 - 30 contacting a biological sample with a reagent which specifically interacts with
the polynucleotide or the galanin receptor-like GPCR polypeptide.

9. A diagnostic kit for conducting the method of any one of claims 6 to 8.

10. A method of screening for agents which decrease the activity of a galanin
5 receptor-like GPCR, comprising the steps of:

contacting a test compound with any galanin receptor-like GPCR polypeptide
encoded by any polynucleotide of claim 1;

10 detecting binding of the test compound to the galanin receptor-like GPCR
polypeptide, wherein a test compound which binds to the polypeptide is
identified as a potential therapeutic agent for decreasing the activity of a
galanin receptor-like GPCR.

15 11. A method of screening for agents which regulate the activity of a galanin
receptor-like GPCR, comprising the steps of:

contacting a test compound with a galanin receptor-like GPCR polypeptide
encoded by any polynucleotide of claim 1; and

20 detecting a galanin receptor-like GPCR activity of the polypeptide, wherein a
test compound which increases the galanin receptor-like GPCR activity is
identified as a potential therapeutic agent for increasing the activity of the
galanin receptor-like GPCR, and wherein a test compound which decreases
25 the galanin receptor-like GPCR activity of the polypeptide is identified as a
potential therapeutic agent for decreasing the activity of the galanin receptor-
like GPCR.

30 12. A method of screening for agents which decrease the activity of a galanin
receptor-like GPCR, comprising the steps of:

contacting a test compound with any polynucleotide of claim 1 and detecting binding of the test compound to the polynucleotide, wherein a test compound which binds to the polynucleotide is identified as a potential therapeutic agent for decreasing the activity of galanin receptor-like GPCR.

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13. A method of reducing the activity of galanin receptor-like GPCR, comprising the steps of:

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Contacting a cell with a reagent which specifically binds to any polynucleotide of claim 1 or any galanin receptor-like GPCR polypeptide of claim 4, whereby the activity of galanin receptor-like GPCR is reduced.

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14. A reagent that modulates the activity of a galanin receptor-like GPCR polypeptide or a polynucleotide wherein said reagent is identified by the method of any of the claim 10 to 12.

20

15. A pharmaceutical composition, comprising:

the expression vector of claim 2 or the reagent of claim 14 and a pharmaceutically acceptable carrier.

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16. Use of the pharmaceutical composition of claim 15 for modulating the activity of a galanin receptor-like GPCR in a disease.
17. Use of claim 16 wherein the disease is pathophysiological disorder, cancer, diabetes, osteoporosis, obesity, pain, depression, ischemia, Alzheimer's disease, sleep disorder, migraine, anxiety, and reproductive disorder.

30

18. Use of the pharmaceutical composition of claim 15 for modulating cognition, analgesia, sensory processing (olfactory, visual), processing or visceral

information, motor coordination, modulation of dopaminergic activity, and neuroendocrine function.

- 5
19. A cDNA encoding a polypeptide comprising the amino acid sequence shown in SEQ ID NO. 2, 4 or 5.
20. The cDNA of claim 19 which comprises of SEQ ID NO. 1 or 3.
- 10
21. The cDNA of claim 19 which consist of SEQ ID NO. 1 or 3.
22. An expression vector comprising a polynucleotide which encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NO. 2, 4 or 5.
- 15
23. The expression vector of claim 22 wherein the polynucleotide consists of SEQ ID NO. 1 or 3.
24. A host cell comprising an expression vector which encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NO. 2, 4 or 5.
- 20
25. The host cell of claim 24 wherein the polynucleotide consists of SEQ ID NO. 1 or 3.
- 25
26. A purified polypeptide comprising the amino acid sequence shown in SEQ ID NO. 2, 4 or 5.
27. The purified polypeptide of claim 26 which consists of the amino acid sequence shown in SEQ ID NO. 2, 4 or 5.
- 30
28. A fusion protein comprising a polypeptide having the amino acid sequence shown in SEQ ID NO. 2, 4 or 5.

29. A method of producing a polypeptide comprising the amino acid sequence shown in SEQ ID NO. 2, 4 or 5, comprising the steps of:

5 culturing a host cell comprising an expression vector which encodes the polypeptide under conditions whereby the polypeptide is expressed; and isolating the polypeptide.

30. The method of claim 29 wherein the expression vector comprises SEQ ID NO. 1 or 3.

- 10 31. A method of detecting a coding sequence for a polypeptide comprising the amino acid sequence shown in SEQ ID NO. 2, 4 or 5, comprising the steps of:

15 hybridizing a polynucleotide comprising 11 contiguous nucleotides of SEQ ID NO. 1 or 3 to nucleic acid material of a biological sample, thereby forming a hybridization complex; and

detecting the hybridization complex.

- 20 32. The method of claim 31 further comprising the step of amplifying the nucleic acid material before the step of hybridizing.

- 25 33. A kit for detecting a coding sequence for a polypeptide comprising the amino acid sequence shown in SEQ ID NO. 2, 4 or 5, comprising:

a polynucleotide comprising 11 contiguous nucleotides of SEQ ID NO. 1 or 3; and

instructions for the method of claim 31.

34. A method of detecting a polypeptide comprising the amino acid sequence shown in SEQ ID NO. 2, 4 or 5, comprising the steps of:

contacting a biological sample with a reagent that specifically binds to the polypeptide to form a reagent-polypeptide complex; and

detecting the reagent-polypeptide complex.

35. The method of claim 34 wherein the reagent is an antibody.

36. A kit for detecting a polypeptide comprising the amino acid sequence shown in SEQ ID NO. 2, 4 or 5 comprising:

an antibody which specifically binds to the polypeptide; and

instructions for the method of claim 34.

37. A method of screening for agents which can regulate the activity of a galanin receptor-like GPCR protein, comprising the steps of:

contacting a test compound with a polypeptide comprising an amino acid sequence selected from the group consisting of: (1) amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO. 2, 4 or 5 and (2) the amino acid sequence shown in SEQ ID NO. 2, 4 or 5; and

detecting binding of the test compound to the polypeptide, wherein a test compound which binds to the polypeptide is identified as a potential agent for regulating activity of the galanin receptor-like GPCR protein.

38. The method of claim 37 wherein the step of contacting is in a cell.

39. The method of claim 37 wherein the cell is in vitro.

40. The method of claim 37 wherein the step of contacting is in a cell-free
5 system.

41. The method of claim 37 wherein the polypeptide comprises a detectable label.

42. The method of claim 37 wherein the test compound comprises a detectable
10 label.

43. The method of claim 37 wherein the test compound displaces a labeled ligand
which is bound to the polypeptide.

15 44. The method of claim 37 wherein the polypeptide is bound to a solid support.

45. The method of claim 37 wherein the test compound is bound to a solid
support.

20 46. A method of screening for agents which regulate an activity of a human
galanin receptor-like GPCR protein, comprising the steps of:

contacting a test compound with a polypeptide comprising an amino acid
sequence selected from the group consisting of: (1) amino acid sequences
25 which are at least about 50% identical to the amino acid sequence shown in
SEQ ID NO. 2, 4 or 5 and (2) the amino acid sequence shown in SEQ ID NO.
2, 4 or 5; and

30 detecting an activity of the polypeptide, wherein a test compound which
increases the activity of the polypeptide is identified as a potential agent for
increasing the activity of the human galanin receptor-like GPCR protein, and

wherein a test compound which decreases the activity of the polypeptide is identified as a potential agent for decreasing the activity of the human galanin receptor-like GPCR protein.

5 47. The method of claim 46 wherein the step of contacting is in a cell.

48. The method of claim 46 wherein the cell is in vitro.

10 49. The method of claim 46 wherein the step of contacting is in a cell-free system.

50. The method of claim 46 wherein the activity is cyclic AMP formation.

15 51. The method of claim 46 wherein the activity is mobilization of intracellular calcium.

52. The method of claim 46 wherein the activity is phosphoinositide metabolism.

20 53. A method of screening for agents which regulate an activity of a human galanin receptor-like GPCR protein, comprising the steps of:

contacting a test compound with a product encoded by a polynucleotide which comprises the nucleotide sequence shown in SEQ ID NO. 1 or 3; and

25 detecting binding of the test compound to the product, wherein a test compound which binds to the product is identified as a potential agent for regulating the activity of the human galanin receptor-like GPCR protein.

30 54. The method of claim 53 wherein the product is a polypeptide.

55. The method of claim 53 wherein the product is RNA.

56. A method of reducing activity of a human galanin receptor-like GPCR protein, comprising the step of:

5 contacting a cell with a reagent which specifically binds to a product encoded by a polynucleotide comprising the nucleotide sequence shown in SEQ ID NO. 1 or 3, whereby the activity of a human galanin receptor-like GPCR protein is reduced.

10 57. The method of claim 56 wherein the product is a polypeptide.

58. The method of claim 57 wherein the reagent is an antibody.

59. The method of claim 56 wherein the product is RNA.

15

60. The method of claim 59 wherein the reagent is an antisense oligonucleotide.

61. The method of claim 59 wherein the reagent is a ribozyme.

20

62. The method of claim 56 wherein the cell is in vitro.

63. The method of claim 56 wherein the cell is in vivo.

64. A pharmaceutical composition, comprising:

25

a reagent which specifically binds to a polypeptide comprising the amino acid sequence shown in SEQ ID NO. 2, 4 or 5; and

a pharmaceutically acceptable carrier.

30

65. The pharmaceutical composition of claim 64 wherein the reagent is an antibody.
- 5 66. A pharmaceutical composition, comprising:
a reagent which specifically binds to a product of a polynucleotide comprising the nucleotide sequence shown in SEQ ID NO. 1 or 3; and
a pharmaceutically acceptable carrier.
- 10 67. The pharmaceutical composition of claim 66 wherein the reagent is a ribozyme.
68. The pharmaceutical composition of claim 66 wherein the reagent is an antisense oligonucleotide.
- 15 69. The pharmaceutical composition of claim 66 wherein the reagent is an antibody.
- 20 70. A pharmaceutical composition, comprising:
an expression vector encoding a polypeptide comprising the amino acid sequence shown in SEQ ID NO. 2, 4 or 5; and
a pharmaceutically acceptable carrier.
- 25 71. The pharmaceutical composition of claim 70 wherein the expression vector comprises SEQ ID NO. 1 or 3.
72. A method of treating pain, comprising the step of:

administering to a patient in need thereof a therapeutically effective dose of a
30 reagent that inhibits a function of a human galanin receptor-like GPCR protein, whereby symptoms of pain are ameliorated.

73. The method of claim 72 wherein the reagent is identified by the method of claim 37.
- 5 74. The method of claim 72 wherein the reagent is identified by the method of claim 46.
75. The method of claim 72 wherein the reagent is identified by the method of claim 53.
- 10 76. The method of claim 72 wherein the disorder is cancer, diabetes, osteoporosis, obesity, depression, ischemia, Alzheimer's disease, sleep disorder, migraine, anxiety, and reproductive disorder.

Fig. 1

TTTTTGAAGT TTTCATTCAT AAATGCATAG ACAATGGGAT TACAGATGGA
GTTGGAAAAT CCAATAATTT GCACGATAGC AAAAATCATC TTGATTGTGA
CATCATCATA TTCCTTTTCA AAATTACTGT ATTCAATCAT CATATGGACA
ACATGGAATG GTGCCCAGCA CACAGCAAAG AGAGCCACCA CTGTCACCAT
CATAATGACA GCTCGTTTCT TCTTCCATAA GAGGCAGGAG GAAGAGGATG
ACAAGGATGA AGGTGGTGTA GATCTTCTGG TGCACAGGGC TGGTCCACTC
TTCTAAGCAG CAGATGTGTT CCTTTTCATA TAGGAAGTCA TATTTGATCT
CAAGTTGTTG CACGTGCCAC ATGGGTGATC CTACGATGAC TGCCACCAGC
CAGACCACAC CTAGCATTGT GAAAGCCCTT CG

Fig. 2

RRAFTMLGVV WLVAIVGVSP MWHVQOLEIK YDFLYEKEHI CCLEEWTSVP
HQKIYTTFIL VILFLLPLM//
KKKRAVIMMV TVVALFAVCW APFHVVHMMI EYSNFEKEYD DVTIKMIFAI
VQIIGFSNSI CNPIVYAFMN ENFK

Fig. 3

TTTGAAGTTT TCATTCATAA ATGCATAGAC AATGGGATTA CAGATGGAGT
TGGAAAATCC AATAATTTGC ACGATAGCAA AAATCATCTT GATTGTGACA
TCATCATATT CCTTTTCAAA ATTACCTGAA ATAAAGTAAT ATTTTAGAAT
GTACGTTTAT TTTAAGGAGT ATAAAATAAA ACTTCTGTTT GTCACCTGTC
AAAGACTTAA ACTTGTAACA GTATCTGAAG GTGTGAAAAG TTCTCAAGAT
CGTTTTGGGA GAGGCATCTG ATATCTGAAT AAAACAACC TTTGTGATGA
GTCTTCACTT AGAGAATCTA GGTTTACATG CGTAGCCTGG TGTCCAGCAT
ATAGTAGGGG GCTTATAGCA TTGCAGCTTG ATAACCTCAA GCTTCATGTA
AACT

Fig. 4

FT*SLELSSCNAISPALLYAGHQATHVNLDLSEDSQRLFLFRYQMPLPK
RS*ELFTPSDTCTSLSL*QVTNRSFILYSLK*TYILKYYFISGNFEKEYD
DVTIKMIFAIVQIIGFSNSICNPIVYAFMNENFK

Fig. 5

MELAMVNLSE GNGSDPEPPA PESRPLFGIG VENFITLVVF GLIFAMGVLG
NSLVITVLAR SKPGKPRSTT NLFILNLSIA DLAYLLFCIP FQATVYALPT
WVLGAFICKF IHYFFTVMML VSIFTLAAMS VDRYVAIVHS RRSSSLRVSR
NALLGVGFIW ALSIAMASPV AYHQRLFHRD SNQTCWEQW PNKLHKKAYV
VCTFVFGYLL PLLLICFCYA KVLNHLHKKL KNMSKKSEAS KKKTAQTVLV
VVVVFGISWL PHHVHLWAE FGAFPLTPAS FFFRITAHCL AYSNSSVNPI
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Fig. 6

Functional Annotation with sequence alignment:
 BLASTP - Query = AI307658_TR; Hit = swissnew|P56479|GALR_MOUSE

This hit is scoring at : 3e-14 (expectation value)
 Alignment length (overlap) : 170
 Identities : 27 %
 Scoring matrix : BLOSUM62 (used to infer consensus pattern)
 Database searched : nrdb

```

Q: 1 RRAFTMLGVVWLVAIVGSPMWHVQQLKIDFLYEKEHICCLEEWTSPVHQKIYTTFFIL
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H: 150 RNALLGVGFIWALSIAMASPVAYHQRL-----FHRDSNQTFCWEQWPNKLNKKAYVVC
      VILFLPLM-----KKKRAVIMMVTVVVVALFAVCWAPFHV
      V. :LLPL:
      VGYLLPLLICFCYAKVLNHLHKKLNMSKKSEASKKTAQTVLVVVVVFGISWLPHHV
      VHMIEYSNFEKEYDDVTIKMIFAI-VQIGFSNSICNPIVYAFMNEFK 143
      VH:..E:. F. ....F.I...:SNS .NPI:YAF:ENF:
      VHLWAEFGAFL-----TPASFFFRITAHCLAYSNSSVNPIIYAFLENFR 310
  
```

Transmembrane domains
 BLOCKS GPCR regions

Transmembrane domains detected by TM-Helix:
 from 6 to 23, from 55 to 72, from 77 to 95, from 119 to 136

BLOCKS search results

AC#	Description	Strength	Score
BL00237D	G-protein coupled receptors proteins.	1222	1270
AA# 126	NSICNPIVYAFMNEFK		
BL00237C	G-protein coupled receptors proteins.	1172	1199
AA# 68	MKKKRAVIMMVTVVVVALFAVCWAPFHV		

Fig. 7

Functional annotation with sequence alignment:

Original EST DNA hits the GALR_MOUSE in minus frame, with a shift:

BLASTX - alignment of 18 against swissnew|P56479|GALR_MOUSE

This hit is scoring at : 8e-14 (expectation value)

Alignment length (overlap) : 74

Identities : 36 %

Scoring matrix : BLOSUM62 (used to infer consensus pattern)

Query reading frame : -2

Database searched : nrdb

Q: 221 KKRAVIMMVTVALFAVCWAPFHVVHMMIEYSNFEKEYDDVTIKMIFAI-VQIIGFSNSI

KK:...:::VV:F::W.P.HVVH:...E::F.....F.I.....:SNS

H: 241 KKKTAAQTVLVVVVVFGISWLPHPHVHVAEFGAFPL----TPASFFFRITAHCLAYSNS

CNPIVYAFMNENFK 3

.NPI:YAF::ENF:

VNPIIYAFLENER 310

This hit is scoring at : 8e-14 (expectation value)

Alignment length (overlap) : 69

Identities : 28 %

Scoring matrix : BLOSUM62 (used to infer consensus pattern)

Query reading frame : -1

Database searched : nrdb

Q: 432 RRAFTMLGVVWLVAIVGSPMWHVQQLEIKYDFLYEKEHICCLEWTSPVHQKIYTFIL

R.A.:G:W:::..SP: : Q:L F :.... C E:W:::H:K.Y... ..

H: 150 RNALLGVGFIWALSIAASPVAYHQRL-----FHRDSNQTFCEQWPNKLHKKAYVVC

VILFLLPLM 226

V.:LLPL:

VFGYLLPLL 213

This target is an EST sequence (AI307658). It has an identical genomic hit (AC005961.1). Here is the exon/intron boundaries:

[illegible]

gi	59390	ATTTTGAAAAGGAATATGATGATGTCACAATCAAGATGATTTTTTGCTATC	59439
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	124	atgtgaaaagggaatatgatgatgtcaccaatcaagatgatttttgctatc	75
gi	59440	GTGCAAAATTATTGGATTTTCCAACCTCCATCTGTAAATCCCATTTGTCTATGC	59489
18			
	74	gtgcaaatatttggaattttccaactccatctgtaatcccattgtctatgc	25
gi	59490	ATTTATGAATGAAAACCTTCAAAAA	59513
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<120> REGULATION OF HUMAN GALANIN RECEPTOR-LIKE G PROTEIN
COUPLED RECEPTOR

<130> Lio063 Foreign Countries

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<150> US 60/205,071

<151> 2000-05-18

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agagccacca ctgtcaccat cataatgaca gctcgtttct tcttcataa gaggcaggag 240
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ttctaagcag cagatgtgtt ccttttcata taggaagtca tatttgatct caagttgttg 360
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Phe Leu Tyr Glu Lys Glu His Ile Cys Cys Leu Glu Glu Trp Thr Ser

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Leu Leu Pro Leu Met
 65

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Pro Lys Arg Ser Xaa Glu Leu Phe Thr Pro Ser Asp Thr Cys Thr Ser
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Lys Glu Tyr Asp Asp Val Thr Ile Lys Met Ile Phe Ala Ile Val Gln
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<213> Homo sapiens

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Ala Ile Val Gln Ile Ile Gly Phe Ser Asn Ser Ile Cys Asn Pro Ile
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Val Tyr Ala Phe Met Asn Glu Asn Phe Lys

65

70

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International Bureau



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15/62, C07K 14/72, C12Q 1/68, G01N 33/50, 33/577,
A61K 38/17, 31/7088, 39/395

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(71) Applicant (for all designated States except US): **BAYER AKTIENGESELLSCHAFT** [DE/DE]; 51368 Leverkusen (DE).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **RAMAKRISHNAN, Shyam** [IN/US]; Apartment #10, 76 Euston Road, Brighton, MA 02135 (US).

(74) Common Representative: **BAYER AKTIENGESELLSCHAFT**; 51368 Leverkusen (DE).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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Published:

— with international search report

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(54) Title: HUMAN GALANIN RECEPTOR-LIKE G PROTEIN COUPLED RECEPTOR

(57) Abstract: Reagents which regulate human galanin receptor-like GPCR and reagents which bind to human galanin receptor-like gene products can be used to regulate the effect of galanin for therapeutic purposes. Treatment of pathophysiological disorders such as eating disorders, cancer, diabetes, osteoporosis, obesity, pain, depression, ischemia, Alzheimer's disease, sleep disorders, migraine, anxiety, and reproductive disorders can be treated. Processes such as cognition, analgesia, sensory processing (olfactory, visual), processing or visceral information, motor coordination, modulation of dopaminergic activity, and neuroendocrine function can be modulated.



WO 01/087930 A3

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/12 C12N15/62 C07K14/72 C12Q1/68 G01N33/50
G01N33/577 A61K38/17 A61K31/7088 A61K39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K C12Q G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL [Online] Entry/Acc.no. AI307658, 11 December 1998 (1998-12-11) STRAUSBERG, R.: "tb27a03.x1 NCI CGAP Kid12 Homo sapiens cDNA clone IMAGE:2055532 3' similar" XP002190600	1-12,15, 16, 19-63, 65,67-71
Y	the whole document	17
Y	BLOOMQUIST B T ET AL: "CLONING AND EXPRESSION OF THE HUMAN GALANIN RECEPTOR GALR2" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, ACADEMIC PRESS INC. ORLANDO, FL, US, vol. 243, 1998, pages 474-479, XP002935073 ISSN: 0006-291X abstract	17

-/--

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

18 February 2002

Date of mailing of the international search report

14 05 2002

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Smalt, R

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BRANCHEK T A T A ET AL: "Galanin receptor subtypes" TRENDS IN PHARMACOLOGICAL SCIENCES, ELSEVIER TRENDS JOURNAL, CAMBRIDGE, GB, vol. 21, no. 3, March 2000 (2000-03), pages 109-117, XP004202574 ISSN: 0165-6147 abstract	
X	--- WO 00 11015 A (ALPHAGENE INC) 2 March 2000 (2000-03-02) see seq.ID's 24 and 25, and passages relating to vc38_1 ---	1-12, 15-19, 22,24, 26,28, 29, 31-63, 65,67-71
X	--- WO 00 22131 A (ARENA PHARMACEUTICALS INC ;GORE MARTIN (US); LIAW CHEN W (US); LIN) 20 April 2000 (2000-04-20) see seq.ID's 39 and 40 and passages relating to hRUP4 ---	1-12, 15-19, 22,24, 26,28, 29, 31-63, 65,67-71
A	--- HABERT-ORTOLI E ET AL: "MOLECULAR CLONING OF A FUNCTIONAL HUMAN GALANIN RECEPTOR" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 91, no. 21, 11 October 1994 (1994-10-11), pages 9780-9783, XP002029082 ISSN: 0027-8424 cited in the application the whole document ---	
A	--- WO 98 29441 A (DOWD BRIAN O ;UNIV TORONTO (CA); KOLAKOWSKI LEE F JR (US); SULLIVA) 9 July 1998 (1998-07-09) the whole document ---	
A	--- WO 98 15570 A (GERALD CHRISTOPHE P G ;BOROWSKY BETH (US); SMITH KELLI E (US); BAR) 16 April 1998 (1998-04-16) the whole document ---	
	--- -/--	

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 01 16316 A (KIKUCHI KUNIKO ;WATANABE TAKUYA (JP); SHINTANI YASUSHI (JP); TAKED) 8 March 2001 (2001-03-08) see seq.ID.2 and passages relating to GPCR AQ27 ---	1-5,19, 22,24, 26,28,29
P,X	WO 00 78809 A (SMITHKLINE BEECHAM CORP) 28 December 2000 (2000-12-28) the whole document ---	1-5,19, 22,24, 26,28, 29, 31-63, 65,67-71
E	WO 01 74897 A (BURGESS CATHERINE E ;SPYTEK KIMBERLY ANN (US); VERNET CORINE A M ()) 11 October 2001 (2001-10-11) see seq.ID's 17, 18, 53 and passages relating to AMF_9 ---	1-12, 15-25, 28, 31-63, 65,67-71
E	WO 01 48189 A (MATSUMOTO SHUN ICHIRO ;MORIKAWA NORIYUKI (JP); SUGIYAMA TOMOYASU ()) 5 July 2001 (2001-07-05) the whole document -----	1-5,19, 22,24, 26,28,29

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 01/05569**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 16-18, in as far as they pertain to in vivo use, are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.: 13,14,64,66,72-76
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-76, all partially

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box 1.2

Claims Nos.: 13,14,64,66,72-76

Present claims 13-18,64,66, and 72-76 relate to products defined by reference to a desirable characteristic or property, namely being able to regulate the activity of the protein(s) of claim 26.

The application provides no support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for any such products. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search impossible. Consequently, claims 13,14,64,66, and 72-76 were not searched, claims 15-18 were searched only in as far as they relate to the expression vector of claim 2.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-76, all partially

Polynucleotide encoding a protein having at least 50% identity to seq.ID.2, expression vector comprising said nucleic acid, host cell comprising said vector, method for producing the protein, the purified protein, methods for detecting the protein or the nucleic acid and kits therefore, methods for screening for compounds which modulate the activity of the protein, use of the xepression vector in a pharmaceutical composition, fusion protein comprising seq.ID.2, pharmaceutical composition comprising an antibody directed against the protein or the nucleic acid, or a ribozyme or antisense oligonucleotide directed against said nucleic acid.

2. Claims: 1-76, all partially

Subject mater as defined under invention 1, but limited to a protein comprising seq.ID.4.

3. Claims: 1-76, all partially

Subject mater as defined under invention 1, but limited to a protein comprising seq.ID.5.

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